part of #16E NE

Trp-R_1-X_7-R_1-R_1-R_2-X-Phe-Phe-Tyr-X-Thr-Glu-X_{8-9}-R_3-R_3-Arg-R_4-X_2-Trp (SEQ ID NOS:11 and 12)

where X is any amino acid and a subscript refers to the number of consecutive residues, R_1 is leucine or isoleucine, R_2 is glutamine or arginine, R_3 is phenylalanine or tyrosine, and R_4 is lysine or histidine. In one embodiment the protein has a sequence of human TRT. In other embodiments, the invention relates to peptides and polypeptides sharing substantial sequence identity with a subsequence of such proteins.

Please replace the paragraph beginning at page 4, line 23, with the following rewritten paragraph:

In a related embodiment the invention provides an isolated, substantially pure or recombinant nucleic acid that encodes a telomerase reverse transcriptase protein. In one embodiment the nucleic acid encodes a protein comprising an amino acid sequence (SEQ ID NOS:11 and 12):

Please replace the paragraph beginning at page 5, line 1, with the following rewritten paragraph:

In one embodiment, the invention relates to human telomerase reverse transcriptase (hTRT) protein. Thus, in one embodiment, the invention provides an isolated, substantially pure, or recombinant protein preparation of an hTRT protein, or a variant thereof, or a fragment thereof. In one embodiment, the protein is characterized by having an amino acid sequence with at least about 75% or at least about 80% sequence identity to the hTRT protein of Figure 17 (SEQ ID NO:2), or a variant thereof, or a fragment thereof. In a related aspect, the hTRT protein has the sequence of SEQ ID NO:2. In some embodiments, the protein has one or more telomerase activities, such as catalytic activity. In one embodiment, the hTRT protein fragment has at least 6 amino acid residues. In other embodiments, the hTRT protein fragment has at least 8, at least about 10, at least about 12, at least about 15 or at least about 20 contiguous amino acid

residues of a naturally occurring hTRT polypeptide. In still other embodiments, the hTRT protein fragment has at least about 50 or at least about 100 amino acid residues.

Please replace the paragraph beginning at page 5, line 24, with the following rewritten paragraph:

In another aspect, the invention provides an isolated, synthetic, substantially pure, or recombinant polynucleotide comprising a nucleic acid sequence that encodes an hTRT protein. In one embodiment, the polynucleotide has a nucleotide sequence encoding an hTRT protein that has an amino acid sequence as set forth in Figure 17 (SEQ ID NO:2) or a sequence that comprises one or more conservative amino acid (or codon) substitutions or one or more activity-altering amino acid (or codon) substitutions in said amino acid sequence. In a related aspect, the polynucleotide hybridizes under stringent conditions to a polynucleotide having the sequence as set forth in Figure 16 (SEQ ID NO:1). In another related aspect, the nucleotide sequence of the polynucleotide has a smallest sum probability of less than about 0.5 when compared to a nucleotide sequence as set forth in Figure 16 (SEQ ID NO:1) using BLAST algorithm with default parameters.

Please replace the paragraph beginning at page 6, line 20, with the following rewritten paragraph:

The invention further provides a method of preparing recombinant telomerase by contacting a recombinant hTRT protein with a telomerase RNA component under conditions such that said recombinant protein and said telomerase RNA component associate to form a telomerase enzyme capable of catalyzing the addition of nucleotides to a telomerase substrate. In one embodiment, the hTRT protein has a sequence as set forth in Figure 17 (SEQ ID NO:2). The hTRT protein may be produced in an *in vitro* expression system and mixed with a telomerase RNA or, in another embodiment, the telomerase RNA can be co-expressed in the *in vitro* expression system. In one embodiment the telomerase RNA is hTR. In an alternative embodiment, the contacting occurs in a cell, such as a human cell. In one embodiment, the cell

does not have telomerase activity prior to the contacting of the hTRT and the RNA, or the introduction, such as by transfection, of an hTRT polynucleotide. In one embodiment, the telomerase RNA is expressed naturally by said cell.

Please replace the paragraph beginning at page 11, line 20, with the following rewritten paragraph:

Figure 1 shows highly conserved residues in TRT motifs from human (SEQ ID NO:13), *S. pombe* (tez1) (SEQ ID NO:14), *S. cerevisiae* (EST2) (SEQ ID NO:15) and *Euplotes aediculatus* (p123) (SEQ ID NO:16). Identical amino acids are indicated with an asterisk (*) [raised slightly], while the similar amino acid residues are indicated by a dot (•). Motif "0" in the figure is also called Motif T; Motif "3" is also called Motif A.

Please replace the paragraph beginning at page 12, line 2, with the following rewritten paragraph:

Figure 4 shows multiple sequence alignment of telomerase RTs (Sp_Trt1p, *S. pombe* TRT (SEQ ID NOS:24-29) [also referred to herein as "tez1p"]; hTRT, human TRT (SEQ ID NOS:30-35) ; Ea_p123, *Euplotes* p123 (SEQ ID NOS:36-41); Sc_Est2p, *S. cerevisiae* Est2p) (SEQ ID NOS:42-48) and members of other RT families (Sc_al, cytochrome oxidase group II intron 1-encoded protein from *S. cerevisiae* mitochondria (SEQ ID NOS:51-56), Dm_TART, reverse transcriptase from Drosophila melanogaster TART non-LTR retrotransposable element) (SEQ ID NOS:57-63; HIV-1, human immunodeficiency virus reverse transcriptase (SEQ ID NOS:64-68)). TRT con (SEQ ID NOS:17-23) and RT con (SEQ ID NOS:49 and 50) represent consensus sequences for telomerase RTs and non-telomerase RTs. Amino acids are designated with an h, hydrophobic; p, polar; c, charged. Triangles show residues that are conserved among telomerase proteins but different in other RTs. The solid line below motif E highlights the primer grip region.

Please replace the paragraph beginning at page 12, line 23, with the following rewritten paragraph:

Figure 11, in two pages, shows an alignment of sequences from four TRT proteins from human (hTRT; SEQ ID NOS:72-79), *S. pombe* Trt1 (spTRT; SEQ ID NOS:80-87), *Euplotes* p123 (Ea_p123; SEQ ID NOS:88-95), and *S. cerevisiae* EST2p TRT (Sc_Est2; SEQ ID NOS:96-104) and identifies motifs of interest. TRT con (SEQ ID NOS:69, 21, 70 and 71) shows a TRT consensus sequence. RT con (SEQ ID NOS:49 and 50) shows consensus residues for other reverse transcriptases. Consensus residues in upper case indicate absolute conservation in TRT proteins.

Please replace the paragraph beginning at page 12, line 27, with the following rewritten paragraph:

Figure 12 shows a Topoisomerase II cleavage site (SEQ ID NO: 108) and NFkB binding site motifs (NFkB_CS1 = SEQ ID NO:105; NFkB-MHC-I.2 = SEQ ID NO:106; NFkB_CS2 = SEQ ID NO:107) in an hTRT intron, with the sequence shown corresponding to SEQ ID NO:7.

Please replace the paragraph beginning at page 12, line 30, with the following rewritten paragraph:

Figure 13, in two pages, shows the sequence of the DNA encoding the *Euplotes* 123 kDa telomerase protein subunit (*Euplotes* TRT; SEQ ID NO:109).

Please replace the paragraph beginning at page 13, line 1, with the following rewritten paragraph:

Figure 14 shows the amino acid sequence of the *Euplotes* 123 kDa telomerase protein subunit (*Euplotes* TRT protein; SEQ ID NO:110).

Please replace the paragraph beginning at page 13, line 3, with the following rewritten paragraph:

Figure 15, in five pages, shows the DNA (SEQ ID NO:111) and amino acid (SEQ ID NO:112) sequences of the *S. pombe* telomerase catalytic subunit (*S. pombe* TRT).

Please replace the paragraph beginning at page 13, line 5, with the following rewritten paragraph:

Figure 16, in two pages, shows the hTRT cDNA sequence, with the sequence shown corresponding to SEQ ID NO:1.

Please replace the paragraph beginning at page 13, line 7, with the following rewritten paragraph:

Figure 17 shows the hTRT protein encoded by the cDNA of Figure 16. The protein sequence shown corresponds to SEQ ID NO:2.

Please replace the paragraph beginning at page 13, line 9, with the following rewritten paragraph:

Figure 18 shows the sequence of clone 712562, with the sequence shown corresponding to SEQ ID NO:3.

Please replace the paragraph beginning at page 13, line 11, with the following rewritten paragraph:

Figure 19 shows a 259 residue protein encoded by clone 712562, with the sequence shown corresponding to SEQ ID NO:10.

Please replace the paragraph beginning at page 13, line 13, with the following rewritten paragraph:

Figure 20 shows, in seven pages, the sequence of a nucleic acid with an open reading frame encoding a Δ182 variant polypeptide, with the sequence shown corresponding to SEQ ID NO:4. This Figure also shows the amino acid sequence of this Δ182 variant polypeptide, with the amino acid sequence shown corresponding to SEQ ID NO:5.

Please replace the paragraph beginning at page 13, line 18, with the following rewritten paragraph:

Figure 21 shows, in six pages, sequence from an hTRT genomic clone, with the sequence shown corresponding to SEQ ID NO:6. Consensus motifs and elements are indicated, including sequences characteristic of a topoisomerase II cleavage site, NFκB binding sites, an Alu sequence and other sequence elements.

Please replace the paragraph beginning at page 13, line 24, with the following rewritten paragraph:

Figure 23 shows the sequence of EST AA281296, corresponding to SEQ ID NO:8.

Please replace the paragraph beginning at page 13, line 26, with the following rewritten paragraph:

Figure 24 shows the sequence of the 182 basepairs deleted in clone 712562, with the sequence shown corresponding to SEQ ID NO:9.

Please replace the paragraph beginning at page 14, line 17, with the following rewritten paragraph:

Figure 32 shows the putative alignments of telomerase RNA template (SEQ ID NO:113), and hairpin primers with telomerase RNA.

Please replace the paragraph beginning at page 14, line 19, with the following rewritten paragraph:

Figure 33 is a photograph of lanes 25-30 of the gel shown in Figure 31, shown at a lighter exposure level ($G_4T_4G_4T_4$ = SEQ ID NO:114).

Please replace the paragraph beginning at page 14, line 21, with the following rewritten paragraph:

Figure 34 shows the DNA sequence of the gene encoding the 43 kDa telomerase protein subunit from *Euplotes* (SEQ ID NO:115).

Please replace the paragraph beginning at page 14, line 23, with the following rewritten paragraph:

Figure 35 shows, in four pages, the DNA sequence (SEQ ID NO:115), as well as the amino acid sequences of all three open reading frames of the 43 kDa telomerase protein subunit from *Euplotes* (a = SEQ ID NOS:116-140; b = SEQ ID NOS:141-162; c = SEQ ID NOS:163-186).

Please replace the paragraph beginning at page 14, line 26, with the following rewritten paragraph:

Figure 36 shows a sequence comparison between the 123 kDa telomerase protein subunit of *Euplotes* (SEQ ID NO:187) (upper sequence) and the 80 kDa polypeptide subunit of *T. thermophila* (SEQ ID NO:188) (lower sequence).

Please replace the paragraph beginning at page 14, line 29, with the following rewritten paragraph:

Figure 37 shows a sequence comparison between the 123 kDa telomerase protein subunit of *E. aediculatus* (SEQ ID NO:189) (upper sequence) and the 95 kDa telomerase polypeptide of *T. thermophila* (SEQ ID NO:190) (lower sequence).

Please replace the paragraph beginning at page 15, line 1, with the following rewritten paragraph:

Figure 38 shows the best-fit alignment between a portion of the "La-domain" of the 43 kDa telomerase protein subunit of *E. aediculatus* (SEQ ID NO:191) (upper sequence) and a portion of the 95 kDa polypeptide subunit of *T. thermophila* (SEQ ID NO:192) (lower sequence).

Please replace the paragraph beginning at page 15, line 4, with the following rewritten paragraph:

Figure 39 shows the best-fit alignment between a portion of the "La-domain" of the 43 kDa telomerase protein subunit of *E. aediculatus* (SEQ ID NO:193) (upper sequence) and a portion of the 80 kDa polypeptide subunit of *T. thermophila* (SEQ ID NO:194) (lower sequence).

Please replace the paragraph beginning at page 15, line 7, with the following rewritten paragraph:

Figure 40 shows the alignment and motifs of the polymerase domain of the 123 kDa telomerase protein subunit of *E. aediculatus* (SEQ ID NOS:38-41) and the polymerase domains of various reverse transcriptases including a cytochrome oxidase group II intron 1-encoded protein from *S. cerevisiae* mitochondria (a1 S.c. (group II)) (SEQ ID NOS:204, 205, 54, 206, and 56), Dong (LINE) (SEQ ID NOS:200-203), and yeast ESTp (L8543.12) (SEQ ID NOS:45, 46, 211 and 212), HIV-RT (SEQ ID NOS:207-210) and consensus (SEQ ID NOS:195-199).

Please replace the paragraph beginning at page 15, line 12, with the following rewritten paragraph:

Figure 41 shows the alignment of a domain of the 43 kDa telomerase protein subunit (SEQ ID NO:213) with various La proteins (human La = SEQ ID NO:214; Xenopus LaA = SEQ ID NO:215; Drosophila La = SEQ ID NO:216; S.c. Lhplp = SEQ ID NO:217).

Please replace the paragraph beginning at page 15, line 16, with the following rewritten paragraph:

Figure 43 shows the amino acid sequence of the *T. thermophila* 80 kDa protein subunit (SEQ ID NO:219).

Please replace the paragraph beginning at page 15, line 18, with the following rewritten paragraph:

Figure 44 shows the nucleotide sequence encoding the *T. thermophila* 95 kDa protein subunit (SEQ ID NO:220).

Please replace the paragraph beginning at page 15, line 20, with the following rewritten paragraph:

Figure 45 shows the amino acid sequence of the *T. thermophila* 95 kDa protein subunit (SEQ ID NO:221).

Please replace the paragraph beginning at page 15, line 22, with the following rewritten paragraph:

Figure 46 shows the amino acid sequence of L8543.12 ("Est2p") (SEQ ID NO:222).

Please replace the paragraph beginning at page 15, line 23, with the following rewritten paragraph:

Figure 47 shows the alignment of the amino acid sequence encoded by the *Oxytricha* PCR product (SEQ ID NO:223) with the *Euplotes* p123 sequence (SEQ ID NO:224).

Please replace the paragraph beginning at page 15, line 25, with the following rewritten paragraph:

Figure 48 shows the DNA sequence of Est2 (SEQ ID NO:225).

Please replace the paragraph beginning at page 15, line 26, with the following rewritten paragraph:

Figure 49 shows partial amino acid sequence from a cDNA clone encoding human telomerase peptide motifs (SEQ ID NO:13).

Please replace the paragraph beginning at page 15, line 28, with the following rewritten paragraph:

Figure 50 shows partial DNA sequence of a cDNA clone encoding human telomerase peptide motifs (SEQ ID NO:8).

Please replace the paragraph beginning at page 15, line 30, with the following rewritten paragraph:

Figure 51 shows the amino acid sequence of *tez1*, also called S. pombe *trt* (SEQ ID NO:112).

Please replace the paragraph beginning at page 16, line 1, with the following rewritten paragraph:

Figure 52 shows, in two pages, the DNA sequence of *tez1* (SEQ ID NO:111). Intronic and other non-coding regions are shown in lower case and exons (*i.e.,* coding regions) are shown in upper case.

Please replace the paragraph beginning at page 16, line 4, with the following rewritten paragraph:

Figure 53 shows the alignment of EST2p (SEQ ID NO:226), *Euplotes* (SEQ ID NO:227), and *Tetrahymena* SEQ ID NO:228) sequences, as well as consensus sequence (SEQ ID NOS:229-231).

Please replace the paragraph beginning at page 16, line 6, with the following rewritten paragraph:

Figure 54 shows the sequences of peptides (SEQ ID NOS:232-237) useful for production of anti-hTRT antibodies.

Please replace the paragraph beginning at page 16, line 9, with the following rewritten paragraph:

Figure 56 shows two degenerate primers (SEQ ID NOS:238 and 241) used in PCR to identify the *S. pombe* homolog of the *E. aediculatus* p123 sequences (SEQ ID NOS:239 and 240).

Please replace the paragraph beginning at page 16, line 11, with the following rewritten paragraph:

Figure 57 shows the four major bands produced in PCR using degenerate primers to identify the *S. pombe* homolog of the *E. aediculatus* p123 sequences (SEQ ID NOS:239 and 240).

Please replace the paragraph beginning at page 16, line 14, with the following rewritten paragraph:

Figure 58 shows the alignment of the M2 PCR product (SEQ ID NO:243) with *E. aediculatus* p123 (SEQ ID NO:242), *S. cerevisiae* (SEQ ID NO:244), and *Oxytricha* (SEQ ID NO:223) telomerase protein sequences. Also shown are the actual genomic sequences (SEQ ID NOS:246 and 249) and the peptides encoded (SEQ ID NOS:245 and 250), degenerate primers Poly4 (SEQ ID NO:238) and Poly 1 (SEQ ID NO:244), and homologous regions of the M2 PCR product (SEQ ID NO:247) and its encoded peptide region (SEQ ID NO:248).

Please replace the paragraph beginning at page 16, line 25, with the following rewritten paragraph:

Figure 63 shows the alignment of RT domains from telomerase catalytic subunits for *S. pombe* (S.p.) (SEQ ID NOS:251-255), *S. cerevisiae* (S.c.) (SEQ ID NOS:256-260) and *E. aediculatus* (E.a.) (SEQ ID NOS:261-265). Consensus sequences = SEQ ID NOS:49 and 50.

Please replace the paragraph beginning at page 16, line 27, with the following rewritten paragraph:

Figure 64 shows the alignment of the sequences from *Euplotes* ("Ea_p123") (SEQ ID NO:110), S. *cerevisiae* ("Sc_Est2p") (SEQ ID NO:222), and *S. pombe* ("SP_Tlplp") (SEQ ID NO:112). In Panel A, the shaded areas indicate residues shared between two sequences. In Panel B, the shaded areas indicate residues shared between all three sequences.

Please replace the paragraph beginning at page 17, line 5, with the following rewritten paragraph:

Figure 68 shows, in four pages, the DNA (SEQ ID NO:266) and amino acid (SEQ ID NO:267) of the ORF encoding an approximately 63 kDa telomerase protein encoded by the EcoRI-NotI insert of clone 712562.

Please replace the paragraph beginning at page 17, line 8, with the following rewritten paragraph:

Figure 69 shows an alignment of reverse transcriptase motifs from various sources, *E aediculatus* p123 (SEQ ID NOS:268-273), *S pombe* tez1 (SEQ ID NOS:274-279), *S. cerevisiae* EST2 (SEQ ID NOS:280-285), and human Hs TCP1 (SEQ ID NOS:286-291), with various consensus residues and motif sequences (SEQ ID NOS:49 and 50) indicated.

Please replace the paragraph beginning at page 17, line 11, with the following rewritten paragraph:

Figure 71 shows, in two pages, the results of preliminary nucleic acid sequencing analysis of a hTRT cDNA sequence (SEQ ID NO:292).

Please replace the paragraph beginning at page 17, line 13, with the following rewritten paragraph:

Figure 72 shows, in ten pages, the preliminary nucleic acid sequence of hTRT (SEQ ID NO:292) and deduced ORF sequences in three reading frames (a = SEQ ID NOS:293-320; b = SEQ ID NOS:321-333; c = SEQ ID NOS:334-342).

Please replace the paragraph beginning at page 17, line 16, with the following rewritten paragraph:

Figure 74 shows, in eight pages, refined nucleic acid sequence (SEQ ID NO:343) and deduced ORF sequences (SEQ ID NO:344) of hTRT

Please replace the paragraph beginning at page 19, line 29, with the following rewritten paragraph:

The *Euplotes* p123, *S. pombe* trt1, and *S. cerevisiae* Est2p nucleic acid sequences of the invention were used in a search of a computerized database of human expressed sequence tags (ESTs) using the program BLAST (Altschul et al. al, 1990, *J. Mol. Biol.* 215:403). Searching this database with the Est2p sequence did not indicate a match, but searching with p123 and trt1 sequences identified a human EST (Genbank accession no. AA281296; see SEQ ID NO:8), as described in Example 1, putatively encoding a homologous protein. Complete sequencing of the cDNA clone containing the EST (hereinafter, Aclone 712562"; see SEQ ID NO:3) showed that seven RT motifs were present. However, this clone did not encode a contiguous human TRT with all seven motifs, because motifs B', C, D, and E were contained in a different open reading frame (ORF) than the more NH_2-terminal motifs. In addition, the distance between motifs A and B' was substantially shorter than that of the three previously characterized TRTs. Clone 712562 was obtained from the I.M.A.G.E. Consortium; Lennon et al., 1996, *Genomics* 33:151.

Please replace the paragraph beginning at page 20, line 12, with the following rewritten paragraph:

A cDNA clone, pGRN121, encoding a functional hTRT (see Figure 16, SEQ ID NO:1) was isolated from a cDNA library derived from the human 293 cell line as described in Example 1. Comparing clone 712562 with pGRN121 showed that clone 712562 has a 182 base pair (see Figure 24, SEQ ID NO:9) deletion between motifs A and B'. The additional 182 base pairs present in pGRN121 place all of the TRT motifs in a single open reading frame, and increase the spacing between the motif A and motif B' regions to a distance consistent with the other known TRTs. As is described *infra* in the Examples (e.g., Example 7), SEQ ID NO:1 encodes a catalytically active telomerase protein having the sequence of SEQ ID NO:2. The polypeptide of SEQ ID NO:2 has 1132 residues and a calculated molecular weight of about 127 kilodaltons (kD).

Please replace the paragraph beginning at page 21, line 1, with the following rewritten paragraph:

Thus, in one aspect, the present invention provides an isolated polynucleotide with a sequence of a naturally occurring human TRT gene or mRNA including, but not limited to, a polynucleotide having the sequence as set forth in Figure 16 (SEQ ID NO:1). In a related aspect, the invention provides a polynucleotide encoding an hTRT protein, fragment, variant or derivative. In another related aspect, the invention provides sense and antisense nucleic acids that bind to an hTRT gene or mRNA. The invention further provides hTRT proteins, whether synthesized or purified from natural sources, as well as antibodies and other agents that specifically bind an hTRT protein or a fragment thereof. The present invention also provides many novel methods, including methods that employ the aforementioned compositions, for example, by providing diagnostic and prognostic assays for human diseases, methods for developing therapeutics and methods of therapy, identification of telomerase-associated proteins, and methods for screening for agents capable of activating or inhibiting telomerase activity. Numerous other aspects and embodiments of the invention are provided *infra*.

Please replace the paragraph beginning at page 22, line 13, with the following rewritten paragraph:

In certain embodiments of the present invention, the hTRT polynucleotides are other than the 389 nucleotide polynucleotide of SEQ ID NO:8 and/or other than clone 712562, the plasmid containing an insert, the sequence of which insert is shown in Figure 18 (SEQ ID NO:3).

Please replace the paragraph beginning at page 23, line 11, with the following rewritten paragraph:

The seven RT motifs found in TRTs, while similar to those found in other reverse transcriptases, have particular hallmarks. For example, as shown in Figure 4, within the TRT RT motifs there are a number of amino acid substitutions (marked with arrows) in residues highly conserved among the other RTs. For example, in motif C the two aspartic acid residues (Defendant) that coordinate active site metal ions (see, Kohlstaedt et al., 1992, *Science* 256:1783; Jacobo-Molina et al., 1993, *Proc. Natl. Acad Sci. U.S.A.* 90:6320; Patel et al., 1995,

Biochemistry 34:5351) occur in the context hxDD(F/Y) (SEQ ID NO:345) in the telomerase RTs compared to (F/Y)xDDh (SEQ ID NO:346) in the other RTs (where "h" is a hydrophobic amino acid, and "x" is any amino acid; see Xiong et al., 1990, *EMBO J.* 9:3353; Eickbush, in *The Evolutionary Biology of Viruses*, (S. Morse, Ed., Raven Press, NY, p. 121, 1994)). Another systematic change characteristic of the telomerase subgroup occurs in motif E, where WxGxSx (SEQ ID NO:347) is a consensus sequence or is conserved among the telomerase proteins, whereas hLGxxh (SEQ ID NO:348) is characteristic of other RTs (Xiong et al., *supra*; Eickbush *supra*). This motif E is called the "primer grip", and mutations in this region have been reported to affect RNA priming but not DNA priming (Powell et al., 1997, *J. Biol. Chem.* 272:13262). Because telomerase requires a DNA primer (e.g., the chromosome 3' end), it is not unexpected that telomerase should differ from other RTs in the primer grip region. In addition, the distance between motifs A and B' is longer in the TRTs than is typical for other RTs, which may represent an insertion within the "fingers" region of the structure which resembles a right hand (Figure 3; see Kohlstaedt et al., *supra*; Jacobo-Molina et al., *supra*; and Patel et al., *supra*).

Please replace the paragraph beginning at page 24, line 2, with the following rewritten paragraph:

Moreover, as noted *supra*, Motif T is an additional hallmark of TRT proteins. This Motif T, as shown, for example in Figure 4 (W-L-X-Y-X-X-h-h-X-h-h-X-p-F-F-Y-X-T-E-X-p-X-X-X-p-X-X-X-Y-X-R-K-X-X-W (SEQ ID NO:349) [X is any amino acid, h is hydrophobic, p is polar]), comprises a sequence that can be described using the formula:

Trp-R_1-X_7-R_1-R_1-R_2-X-Phe-Phe-Tyr-X-Thr-Glu
-X_{8-9}-R_3-R_3-Arg-R_4-X_2-Trp (SEQ ID NOS:11 and 12)

where X is any amino acid and the subscript refers to the number of consecutive residues, R_1 is leucine or isoleucine, R_2 is glutamine or arginine, R_3 is phenylalanine or tyrosine, and R_4 is lysine or histidine.

Please replace the paragraph beginning at page 24, line 12, with the following rewritten paragraph:

The T motif can also be described using the formula:

Trp-R_1-X_4-h-h-X-h-h-R_2-p-Phe-Phe-Tyr-X-Thr-Glu-
X-p-X_3-p-X_{2-3}- R_3-R_3-Arg-R_4-X_2-Trp (SEQ ID NOS:350 and 351)

where X is any amino acid and a subscript refers to the number of consecutive residues, R_1 is leucine or isoleucine, R_2 is glutamine or arginine, R_3 is phenylalanine or tyrosine, R_4 is lysine or histidine, h is a hydrophobic amino acid selected from Ala, Leu, Ile, Val, Pro, Phe, Trp, and Met, and p is a polar amino acid selected from Gly, Ser, Thr, Tyr, Cys, Asn and Gln.

Please replace the paragraph beginning at page 24, line 20, with the following rewritten paragraph:

In one embodiment, the present invention provides isolated naturally occurring and recombinant TRT proteins comprising one or more of the motifs illustrated in Figure 11, e.g.,

Motif T	W-X_{12}-FFY-X-TE-X_{10-11}-R-X_3-W-X_7-I (SEQ ID NOS:352 and 353)
Motif T'	E-X_2-V-X (SEQ ID NO:354)
Motif 1	X_3-R-X_2-P-K-X_3, (SEQ ID NO:355) or, alternatively, h-R-h-X-P-K (SEQ ID NO:633)
Motif 2	X-R-X-I-X (SEQ ID NO:356) or, alternatively, (F/L)-R-h-I-X_2-h (SEQ ID NO:634)
Motif A	X_4-F-X_3-D-X_4-YD-X_2, (SEQ ID NO:357) or, alternatively, P-X-L-Y-F-h-X-h-D-h-X_2-C-Y-D-X-I (SEQ ID NO:635)
Motif B'	Y-X_4-G-X_2-QG-X_3-S-X_8 (SEQ ID NO:358) or, alternatively, K-X-Y-X-Q-X_2-G-I-P-Q-G-S-X-L-S-X-h-L (SEQ ID NO:636)
Motif C	X_6-DD-X-L-X_3, (SEQ ID NO:359) or, alternatively, L-L-R-L-X-D-D-X-L-h-I-T (SEQ ID NO:637)

When the TRT protein shown contains more than one TRT motif, the order (NH2 ->COOH) is as shown in Figure 11.

Please replace the paragraph beginning at page 25, line 11, with the following rewritten paragraph:

It will be apparent to one of skill that, provided with the reagents, including the TRT sequences disclosed herein for those reagents and the methods and guidance provided herein (including specific methodologies described *infra*), TRT genes and proteins can be obtained, isolated and produced in recombinant form by one of ordinary skill. For example, primers (e.g., degenerate amplification primers) are provided that hybridize to gene sequences encoding RT and T motifs characteristic of TRT. For example, one or more primers or degenerate primers that hybridize to sequences encoding the FFYXTE (SEQ ID NO:360) region of the T motif, other TRT motifs (as discussed *infra*), or combinations of motifs or consensus sequences, can be prepared based on the codon usage of the target organism, and used to amplify the TRT gene sequence from genomic DNA or cDNA prepared from the target organism. Use of degenerate primers is well known in the art and entails use of sets of primers that hybridize to the set of nucleic acid sequences that can potentially encode the amino acids of the target motif, taking into account codon preferences and usage of the target organism, and by using amplification (e.g., PCR) conditions appropriate for allowing base mismatches in the annealing steps of PCR. Typically two primer sets are used; however, single primer (or, in this case, a single degenerate primer set) amplification systems are well known and may be used to obtain TRT genes.

Please replace the Table beginning at page 26, line 3, with the following rewritten table:

TABLE 1

ILLUSTRATIVE DEGENERATE PRIMERS FOR AMPLIFICATION OF TRT NUCLEIC ACIDS

	motif	motif SEQ ID NO:	direction	5' sequence –3'	primer SEQ ID NO:
a	FFYVTE	361	Forward	TT(CT)TT(CT)TA(CT)GTNACNGA	362
b	FFYVTE	361	Reverse	TCNGTNAC(GA)TA(GA)AA(GA)AA	363
c	RFIPKP	364	Forward	(CA)GNTT(CT)AT(ACT)CCNAA(AG)CC	365
d	RFIPKP	364	Reverse	GG(TC)TTNGG(TGA)AT(GA)AANC	366
e	AYDTI	367	Forward	GCNTA(CT)GA(CT)ACNAT	368
f	AYDTI	367	Reverse	TANGT(GA)TC(GA)TANGC	369
g	GIPQG	370	Forward	GGNAT(ACT)CCNCA(AG)GG	371
h	GIPQGS	21	Reverse	(GC)(AT)NCC(TC)TGNGG(TGA)ATNCC	372
i	LVDDFL	373	Forward	(CT)TNGTNGA(CT)GA(CT)TT(CT)(CT)T	374
j	DDFLLVT	375	Reverse	GTNACNA(GA)NA(GA)(GA)AA(GA)TC(GA)TC	376

Please replace the paragraph beginning at page 27, line 7, with the following rewritten paragraph:

It will also be apparent to those of skill that TRT genes may be cloned using any of a variety of cloning methods of the invention because the TRT motif sequences and the nucleic acids of the invention comprising such sequences can be used in a wide variety of such methods. For example, hybridization using a probe based on the sequence of a known TRT to DNA or other nucleic acid libraries from the target organism, as described in Example 1 can be used. It will be appreciated that degenerate PCR primers or their amplification products such as those described *supra,* may themselves be labeled and used as hybridization probes. In another embodiment, expression cloning methods are used. For example, one or more antibodies that specifically bind peptides that span a TRT motif or other TRT epitope, such as the FFYXTE

(SEQ ID NO:360) motif can be employed to isolate a ribosomal complex comprising a TRT protein and the mRNA that encodes it. For generating such antibodies of the invention, the peptide immunogens are typically between 6 and 30 amino acids in length, more often about 10 to 20 amino acids in length. The antibodies may also be used to probe a cDNA expression library derived from the organism of interest to identify a clone encoding a TRT sequence. In another embodiment, computer searches of DNA databases for DNAs containing sequences conserved with known TRTs can also be used to identify a clone comprising TRT sequence.

Please replace the paragraph beginning at page 28, line 27, with the following rewritten paragraph:

In one aspect, the invention provides a polynucleotide having a sequence or subsequence of a human TRT gene or RNA. In one embodiment, the polynucleotide of the invention has a sequence of SEQ ID NO: 1 shown in Figure 16 or a subsequence thereof. In another embodiment, the polynucleotide has a sequence of SEQ ID NO:3 (Figure 18), SEQ ID NO:4 (Figure 20), or subsequences thereof. The invention also provides polynucleotides with substantial sequence identity to the hTRT nucleic acid sequences disclosed herein, e.g., including but not limited to SEQ ID NOS:1 [Figure 16], 4 [Figure 20], 6 [Figure 21], and 7 [Figure 12]). Thus, the invention provides naturally occurring alleles of human TRT genes and variant polynucleotide sequences having one or more nucleotide deletions, insertions or substitutions relative to an hTRT nucleic acid sequence disclosed herein. As described *infra*, variant nucleic acids may be produced using the recombinant or synthetic methods described below or by other means.

Please replace the paragraph beginning at page 29, line 9, with the following rewritten paragraph:

The invention also provides isolated and recombinant polynucleotides having a sequence from a flanking region of a human TRT gene. Such polynucleotides include those derived from genomic sequences of untranslated regions of the hTRT mRNA. An exemplary genomic

sequence is shown in Figure 21 (SEQ ID NO:6). As described in Example 4, SEQ ID NO:6 was obtained by sequencing a clone, λGΦ5 isolated from a human genomic library. Lambda GΦ5 contains a 15 kilobasepair (kbp) insert including approximately 13,000 bases 5' to the hTRT coding sequences. This clone contains hTRT promoter sequences and other hTRT gene regulatory sequences (e.g., enhancers).

Please replace the paragraph beginning at page 29, line 23, with the following rewritten paragraph:

In a related aspect, the present invention provides polynucleotides that encode hTRT proteins or protein fragments, including modified, altered and variant hTRT polypeptides. In one embodiment, the encoded hTRT protein or fragment has an amino acid sequence as set forth in Figure 17 (SEQ ID NO:2), or with conservative substitutions of SEQ ID NO:2. In one embodiment, the encoded hTRT protein or fragment has substitutions that change an activity of the protein (e.g., telomerase catalytic activity).

Please replace the paragraph beginning at page 29, line 30, with the following rewritten paragraph:

It will be appreciated that, as a result of the degeneracy of the genetic code, the nucleic acid encoding the hTRT protein need not have the sequence of a naturally occurring hTRT gene, but that a multitude of polynucleotides can encode an hTRT polypeptide having an amino acid sequence of SEQ ID NO:2. The present invention provides each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices made in accordance with known triplet genetic codes, and all such variations are specifically disclosed hereby. Thus, although in some cases hTRT polypeptide-encoding nucleotide sequences that are capable of hybridizing to the nucleotide sequence of the naturally occurring sequence (under appropriately selected conditions of stringency) are preferred, it may be advantageous in other cases to produce nucleotide sequences encoding hTRT that employ a

substantially different codon usage and so perhaps do not hybridize to nucleic acids with the naturally occurring sequence.

Please replace the paragraph beginning at page 30, line 12, with the following rewritten paragraph:

In particular embodiments, the invention provides hTRT oligo- and polynucleotides that comprise a subsequence of an hTRT nucleic acid disclosed herein (e.g., SEQ ID NOS:1 and 6). The nucleic acids of the invention typically comprise at least about 10, more often at least about 12 or about 15 consecutive bases of the exemplified hTRT polynucleotide. Often, the nucleic acid of the invention will comprise a longer sequence, such as at least about 25, about 50, about 100, about 200, or at least about 500 to 3000 bases in length, for example when expression of a polypeptide, or full length hTRT protein is intended.

Please replace the paragraph beginning at page 30, line 20, with the following rewritten paragraph:

In still other embodiments, the present invention provides "Δ182 Htrt" polynucleotides having a sequence identical or complementary to naturally occurring or non-naturally occurring hTRT polynucleotides such as SEQ ID NO:3 or SEQ ID NO:4, which do not contain the 182 nucleotide sequence (SEQ ID NO:9) found in pGRN121 (and also absent in clone 712562). These polynucleotides are of interest, in part, because they encode polypeptides that contain different combinations or arrangements of TRT motifs than found in the "full-length" hTRT polypeptide (SEQ ID NO:2) such as is encoded by pGRN121. As discussed *infra*, it is contemplated that these polypeptides may play a biological role in nature (e.g., in regulation of telomerase expression in cells) and/or find use as therapeutics (e.g., as dominant-negative products that inhibit function of wild-type proteins), or have other roles and uses, e.g. as described herein.

Please replace the paragraph beginning at page 31, line 1, with the following rewritten paragraph:

For example, in contrast to the polypeptide encoded by pGRN121, clone 712562 encodes a 259 residue protein with a calculated molecular weight of approximately 30 kD (hereinafter, "712562 hTRT"). The 712562 hTRT polypeptide (SEQ ID NO:10) contains motifs T, 1, 2, and A, but not motifs B', C, D and E (See Figure 4). Similarly, a variant hTRT polypeptide with therapeutic and other activities may be expressed from a nucleic acid similar to the pGRN121 cDNA but lacking the 182 basepairs missing in clone 712562, e.g., having the sequence shown in Figure 20 (SEQ ID NO:4). This nucleic acid (hereinafter, "pro90 hTRT"), which may be synthesized using routine synthetic or recombinant methods as described herein, encodes a protein of 807 residues (calculated molecular weight of approximately 90 kD) that shares the same amino terminal sequence as the hTRT protein encoded by SEQ ID NO:1, but diverges at the carboxy-terminal region (the first 763 residues are common, the last 44 residues of pro90 hTRT are different than "full-length" hTRT). The pro90 hTRT polypeptide contains motifs T, 1, 2, and A, but not motifs B, C, D, E, and thus may have some, but not likely all telomerase activities.

Please replace the paragraph beginning at page 32, line 3, with the following rewritten paragraph:

In one embodiment, hTRT genes or cDNAs are cloned using a nucleic acid probe that specifically hybridizes to an hTRT mRNA, cDNA, or genomic DNA. One suitable probe for this purpose is a polynucleotide having all or part of the sequence provided in Figure 16 (SEQ ID NO:1), such as a probe comprising a subsequence thereof. Typically, the target hTRT genomic DNA or cDNA is ligated into a vector (e.g., a plasmid, phage, virus, yeast artificial chromosome, or the like) and may be isolated from a genomic or cDNA library (e.g., a human placental cDNA library). Once an hTRT nucleic acid is identified, it can be isolated according to standard methods known to those of skill in the art. An illustrative example of screening a human cDNA library for the hTRT gene is provided in Example 1; similarly, an example of screening a human genomic library is found in Examples 3 and 4. Cloning methods are well known and are described, for example, in Sambrook et al., (1989) MOLECULAR CLONING: A LABORATORY

MANUAL, 2ND ED., VOLS. 1-3, Cold Spring Harbor Laboratory hereinafter, "Sambrook"); Berger and Kimmel, (1987) METHODS IN ENZYMOLOGY, VOL. 152: GUIDE TO MOLECULAR CLONING TECHNIQUES, San Diego: Academic Press, Inc.; Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing and Wiley-Interscience, New York (1997); Cashion et al., U.S. Patent No. 5,017,478; and Carr, European Patent No. 0,246,864.

Please replace the paragraph beginning at page 32, line 21, with the following rewritten paragraph:

The invention also provides hTRT genomic or cDNA nucleic acids isolated by amplification methods such as the polymerase chain reaction (PCR). In one embodiment, hTRT protein coding sequence is amplified from an RNA or cDNA sample (e.g., double stranded placental cDNA (Clontech, Palo Alto CA)) using the primers 5'-GTGAAGGCACTGTTCAGCG-3' ("TCP1.1") (SEQ ID NO:377) and 5'-CGCGTGGGTGAGGTGAGGTG-3 ("TCP 1.15") (SEQ ID NO:378). In some embodiments a third primer or second pair of primers may be used, e.g., for "nested PCR", to increase specificity. One example of a second pair of primers is 5'-CTGTGCTGGGCCTGGACGATA-3' ("TCP1.14") (SEQ ID NO:379) and 5'-AGCTTGTTCTCCATGTCGCCGTAG-3' ("billTCP6") (SEQ ID NO:380). It will be apparent to those of skill that numerous other primers and primer combinations, useful for amplification of hTRT nucleic acids are provided by the present invention.

Please replace the paragraph beginning at page 33, line 2, with the following rewritten paragraph:

Moreover, the invention provides primers that amplify any specific region (e.g., coding regions, promoter regions, and/or introns) or subsequence of hTRT genomic DNA, cDNA or RNA. For example, the hTRT intron at position 274/275 of SEQ ID NO:1 (see Example 3) may be amplified (e.g., for detection of genomic clones) using primers TCP1.57 and TCP1.52 (primer pair 1) or primers TCP1.49 and TCP1.50 (primer pair 2). (Primer names refer to primers

listed in Table 2, *infra.*) The primer pairs can be used individually or in a nested PCR where primer set 1 is used first. Another illustrative example relates to primers that specifically amplify and so detect the 5' end of the hTRT mRNA or the exon encoding the 5' end of hTRT gene (e.g., to assess the size or completeness of a cDNA clone). The following primer pairs are useful for amplifying the 5' end of hTRT: primers K320 and K321 (primer pair 3); primers K320 and TCP1.61 (primer pair 4); primers K320 and K322 (primer pair 5). The primer sets can be used in a nested PCR in the order set 5, then set 4 or set 3, or set 4 or set 5, then set 3. Yet another illustrative example involves primers chosen to amplify or detect specifically the conserved hTRT TRT motif region comprising approximately the middle third of the mRNA (e.g., for use as a hybridization probe to identify TRT clones from, for example, nonhuman organisms). The following primer pairs are useful for amplifying the TRT motif region of hTRT nucleic acids: primers K304 and TCP1.8 (primer pair 6), or primers Lt1 and TCP1.15 (primer pair 7). The primer sets can be used in a nested PCR experiment in the order set 6 then set 7.

Please replace the paragraph beginning at page 35, line 6, with the following rewritten paragraph:

As noted *supra*, the present invention provides nucleic acids having flanking (5' or 3') and intronic sequences of the hTRT gene. The nucleic acids are of interest, *inter alia*, because they contain promoter and other regulatory elements involved in hTRT regulation and useful for expression of hTRT and other recombinant proteins or RNA gene products. It will be apparent that, in addition to the nucleic acid sequences provided in SEQ ID NOS:6 and 7, additional hTRT intron and flanking sequences may be readily obtained using routine molecular biological techniques. For example, additional hTRT genomic sequence may be obtained from Lambda clone GΦ5 (ATCC Accession No. 209024), described *supra* and in Example 4. Still other hTRT genomic clones and sequences may be obtained by screening a human genomic library using an hTRT nucleic acid probe having a sequence or subsequence from SEQ ID NO:1. Additional clones and sequences (e.g., still further upstream) may be obtained by using labeled sequences or subclones derived from λGΦ5 to probe appropriate libraries. Other useful methods for further

characterization of hTRT flanking sequences include those general methods described by Gobinda et al., 1993, *PCR Meth. Applic.* 2:318; Triglia et al., 1988, *Nucleic Acids Res.* 16:8186; Lagerstrom et al., 1991, *PCR Methods Applic.* 1:111; and Parker et al., 1991, *Nucleic Acids Res.* 19:3055.

Please replace the paragraph beginning at page 36, line 11, with the following rewritten paragraph:

The 5' untranslated sequences of hTRT or other TRT mRNAs can be determined directly by cloning a "full-length" hTRT or other cDNA using standard methods such as reverse transcription of mRNA, followed by cloning and sequencing the resulting cDNA. Preferred oligo(dT)-primed libraries for screening or amplifying full length cDNAs that have been size-selected to include larger cDNAs may be preferred. Random primed libraries are also suitable and often include a larger proportion of clones that contain the 5' regions of genes. Other well known methods for obtaining 5' RNA sequences, such as the RACE protocol described by Frohman et al., 1988, *Proc. Nat. Acad. Sci USA* 85:8998, may also be used. If desired, the transcription start site of an hTRT or other TRT mRNA can be determined by routine methods using the nucleic acids provided herein (e.g., having a sequence of SEQ ID NO:1). One method is S1 nuclease analysis (Ausubel et al., *supra*) using a labeled DNA having a sequence from the 5' region of SEQ ID NO:1.

Please replace the paragraph beginning at page 39, line 26, with the following rewritten paragraph:

In Table 2, "seq" means that the primer has been used, or is useful, for sequencing; "PCR" means that the primer has been used, or is useful, for PCR; "AS" means that means that the primer has been used, or is useful for antisense inhibition of telomerase activity; "CL" means that the primer has been used, or is useful in cloning regions of hTRT genes or RNA, "mut" means that the primer has been used, or is useful for constructing mutants of hTRT genes or gene products. "University of California," means "upper case," and "lc" means "lower case."

Mismatches and insertions (relative to SEQ ID NO:1) are indicated by underlining; deletions are indicated by a "-". It will be appreciated that nothing in Table 2 is intended to limit the use of any particular oligonucleotide to any single use or set of uses.

TABLE 2
USEFUL OLIGONUCLEOTIDES

primer	5'-sequence-3' *	Notes	mismatch? *	seq	USE PCR	AS	CL	MUT	SEQ ID NO:
TCP1.1	GTGAAGGCACTGTTCAGCG			x	x				377
TCP1.2	GTGGATGATTTCTTGTTGG			x	x				381
TCP1.4	CTGGACACTCAGCCCTTGG			x	x				382
TCP1.5	GGCAGGTGTGCTGGACACT			x	x				383
TCP1.6	TTTGATGATGCTGGCGATG			x	x				384
TCP1.7	GGGGCTCGTCTTCTACAGG		Y	x	x				385
TCP1.8	CAGCAGGAGGATCTTGTAG			x	x				386
TCP1.9	TGACCCCAGGAGTGGCACG			x	x				387
TCP1.10	TCAAGCTGACTCGACACCG			x	x				388
TCP1.11	CGGCGTGACAGGGCTGC			x	x				389
TCP1.12	GCTGAAGGCTGAGTGTCC			x	x				390
TCP1.13	TAGTCCATGTTCACAATCG			x	x				391
TCP1.14	CTGTGCTGGGCCTGGACGATA			x	x				379
TCP1.15	CGCGTGGGTGAGGTGAGGTG			x	x				378
TCP1.16	TTTCCGTGTTGAGTGTTTC			x	x				392
TCP1.17	GTCACCGTGTTGGGCAGG			x	x				393
TCP1.19	GCTACCTGCCCAACACGG			x	x				394
TCP1.20	GCGCGAAGAACGTGCTGG			x	x				395
TCP1.21	CA-CTGCTCCTTGTCGCCTG		Y	x	x				396
TCP1.22	TTCCCAAGGACTTTGTTGC			x	x				397
TCP1.24	TGTTCCTCAAGACGCACTG		Y	x	x				398
TCP1.25	TACTGCGTGCGTCGGTATG			x	x				399
TCP1.26	GGTCTTGCGGCTGAAGTGT			x	x				400
TCP1.27	TGGTTCACCTGCTGGCACG			x	x				401
TCP1.28	GTGGTTTCTGTGTGGTGTC			x	x				402
TCP1.29	GACACCACACAGAAACCAC			x	x				403
TCP1.30	GTGCCAGCAGGTGAACCAG			x	x				404
TCP1.32B	GCAGTGCGTCTTGAGGAGC			x	x				405
TCP1.33	TGGAACCATAGCGTCAGGGAG			x	x				406
TCP1.34	GGCCTCCCTGACGCTATGGTT			x	x				407
TCP1.35	GC(GT)CGGCGCTGCCACTCAGG			x	x				408
TCP1.35t	GCTCGGCGCTGCCACTCAGG								409
TCP1.36	ACGCCGAGACCAAGCACTTC			x	x				410
TCP1.38	CCAAAGAGGTGGCTTCTTCG			x	x				411
TCP1.39	AAGGCCAGCACGTTCTTCGC			x	x				412
TCP1.40	CACGTTCGTGCGGCGCCTG			x	x				413
TCP1.41	CCTTCACCACCAGCGTGCG			x	x				414
TCP1.42	GGCGACGACGTGCTGGTTC			x	x				415
TCP1.43	GGCTCAGGGGCAGCGCCAC			x	x				416

TCP1.44	CTGGCAGGTGTACGGCTTC			x	x		417
TCP1.45	GCGTGGACCGAGTGACCGTGGTTTC			x	x		418
TCP1.46	GACGTGGTGGCCGCGATGTGG			x	x		419
TCP1.47	GAAGTCTGCCGTTGCCCAAGAG			x	x		420
TCP1.48	GACACCACACAGAAACCACGGTCAC			x	x		421
TCP1.49	CGCCCCCTCCTTCCGCCAGGT			x	x		422
TCP1.50	CGAAGCCGAAGGCCAGCACGTTCTT			x	x		423
TCP1.51	GGTGGCCCGAGTGCTGCAGAGG			x	x		424
TCP1.52	GTAGCTGCGCACGCTGGTGGTGAAG			x	x		425
TCP1.53	TGGGCGACGACGTGCTGGTTCA			x	x		426
TCP1.54	TATGGTTCCAGGCCCGTTCGCATCC			x	x		427
TCP1.55	CCAGCTGCGCCTACCAGGTGTGC			x	x		428
TCP1.56	GGCCTCCCTGACGCTATGGTTCCAG			x	x		429
TCP1.57	GGTGCTGCCGCTGGCCACGTTCG			x	x		430
TCP1.58	TCCCAGGGCACGCACACCAGGCACT			x	x		431
TCP1.59	GTACAGGGCACACCTTTGGTCACTC			x	x		432
TCP1.60	TCGACGACGTACACACTCATCAGCC			x	x		433
TCP1.61	AGCGGCAGCACCTCGCGGTAGTGGC			x	x		434
TCP1.62	CCACCAGCTCCTTCAGGCAGGACAC			x	x		435
TCP1.63	CCAGGGCTTCCCACGTGCGCAGCAG			x	x		436
TCP1.64	CGCACGAACGTGGCCAGCGGCAGCA			x	x		437
TCP1.65	TGACCGTGGTTTCTGTGTGGTGT			x	x		438
TCP1.66	CCCTCTTCAAGTGCTGTCTGATTCC			x	x		439
TCP1.67	ATCGCGGCCACCACGTCCCT			x	x		440
TCP1.68	TGCTCCAGACACTCGGCCGGTAGAA			x	x		441
TCP1.69	ACGAAGCCGTACACCTGCC			x	x		442
TCP1.72	CGACATCCCTGCGTTCTTGGCTTTC			x	x		443
TCP1.73	CACTGCTGGCCTCATTCAGGG			x	x		444
TCP1.74	GCGACATGGAGAACAAGC			x	x		445
TCP1.75	GCAGCCATACTCAGGGACAC			x	x		446
TCP1.76	CCATCCTCTCCACGCTGCTC			x	x		447
TCP1.77	GCGATGACCTCCGTGAGCCTG			x	x		448
TCP1.78	CCCAGGACAGGCTCACGGA			x	x		449
billTCP1	CCTCTTCAAGTGCTGTCTGATTCC			x	x		450
billTCP2	CAGCTCGACGACGTACACACTCATC			x	x		451
billTCP4	CTGACGTCCAGACTCCGCTTCAT			x	x		452
billTCP6	AGCTTGTTCTCCATGTCGCCGTAG			x	x		380
rpprim01	GACCTGAGCAGCTCGACGACGTACACACTCATC			x	x		453
Lt1	GTCGTCGAGCTGCTCAGGTC			x	x		454
Lt2	AGCACGCTGAACAGTGCCTT			x	x		455
Lt3	GACCTGAGCAGCTCGACGAC			x	x		456
Lt4	AAGGCACTGTTCAGCGTGCT			x	x		457
Lt5	CGGCCGAGTGTCTGGAGCAA		Y	x	x		458
Lt6	GGATGAAGCGGAGTCTGGA			x	x		459
BamH1Lt7	ATGGATCCGTCGTCGAGCTGCTCAGGTCT	BamH1 site	Y		x	x	460

Sal1Lt8	ATCAGC TGAGCACGCTGAACAGTGCCTTC	Pvu II site (not Sal I)	Y		x	x	461
K303	GTCTCCGTGACATAAAAGAAAGAC			x	x		462
K304	GCCAAGTTCCTGCACTGGCT			x	x		463
K305	GCCTGTTCTTTTGAAACGTGGTCT			x	x		464
K306	XXGCCTGTTCTTTTGAAACGTGGTCT	X = biotin, = K305		x	x		465
K311	GTCAAGATGCCTGAGATAGAAC			x	x		466
K312	TGCTTAGCTTGTGGGGGTGTCA			x	x		467
K313	TGCTTAGCTTGTGGGGGTGTCA			x	x		467
K320	GCTGCGTCCTGCTGCGCACGT			x	x		468
K321	CAGCGGGGAGCGCGCGGCATC			x	x		469
K322	TGGGCCACCAGCGCGCGGAAA			x	x		470
slanti.1	CGGCCGCAGCCCGTCAGGCTTGGGG		Y	x	x		471
slanti.2	CCGACAGCTCCCGCAGCTGCACCC		Y	x	x		472
slanti.3	CGTACACACTCATCAGCCAGTGCAGGAACTTGGC			x	x		473
slanti.4	CGCGCCCGCTCGTAGTTGAGCACGCTGAACAGTGCCTTC ACCCTCG			x	x		474
slanti.5	GCGGAGTCTGGACGTCAGCAGGGCGGGCCTGGCTTCCCG			x	x		475
UTR2	ATTTGACCCACAGGGACCCCCATCCAG			x	x		476
FW5	ATGACCGCCCTCCTCGTGAG			x	x		477
Nam1	GCCACCCCCGCGATGCC			x	x		478
Nam2	AGCCCTGGCCCCGGCCA			x	x		479
Nam3	TCCCACGTGCGCAGCAG			x	x		480
Nam4	AGCAGGACGCAGCGCTG			x	x		481
PE01	CGCGGTAGTGGCTGCGCAGCAGGGAGCGCACGGC			x	x		482
PE02	CCAGGGCTTCCCACGTGCGCAGCAGGACGCAGCGC			x	x		483
LM101	CTAGTCTAGATCA / GCTAGCGTAATCTGGAACATCGTA TGGGTA / GTCCAGGATGGTCTTGAAGTC	Xba I site / HA tag / hTRT into pGRN121				x	484
LM103	TACCATGGGCTACCCATACGACGTTCCAGATTACGCTCA	inserts HA tag into a Nde I site at 5' end of hTRT				x	485
LM104	TATGAGCGTAATCTGGAACGTCGTATGGGTAGCCCATGG	anneals to LM103				x	486
LM105	GTGTACGTCGTCGAGCTCCTCAGGTCTGCCTTTT ATGTCACGGAG	change = F560A (phe > ala)				x	487
LM106	GTGTACGTCGTCGAGCTCCTCAGGTCTTTCGCTTATGTC ACGGAGACC	change = F561A (phe > ala)				x	488
LM107	CCTCAGGTCTTTCTTTGCTGTCACGGAGACAACGTTT CAAAAGAACAG	change = Y562A (tyr > ala)				x	489

LM108	GGTCTTTCTTTTATGTCGCGGAGACAACGTTT CAAAAGAACAG	change = T564A (thr > ala)				x	490
LM109	CTTTCTTTTATGTCACGGCGACAACGTTTCAAAAGAACA G	change = E565A				x	491
LM_FFYTE	ATGAGTGTGTACGTCGTCGAGCTCCTCAGGTCTACCACG TTTCAAAAGAACAGGCTCTTTTC	deletion of FFYVTE (aa560-565)				x	492
TCP061:	GGCTGATGAGTGTGTACGTCGTCGA	complement to TCP1.61	x	x			493
HUMO1:	ACGTGGTCTCCGTGACATAAAAGAA	to DD motif, designed to possibly anneal to mTRT	x	x	x		494
HUMO2:	AGGTCTTTCTTTTATGTCACGGA	to DD motif, designed to possibly anneal to mTRT	x	x	x		495
HUMO3:	CACAGACCCCCGTCGCCTGGTC	designed to possibly anneal to mTRT	x	x	x		496
HUMO4:	CGGAGTCTGGACGTCAGCAGGGC	designed to possibly anneal to mTRT	x	x	x		497
SLW F1N	cgcggatccgtaactaaaATGCCGCGCGCTCCCCGCTGC	for GST fusion construct (782 to 1636) UC = hTRT seq, lc = BamH1 site + 2 stop codons		x	x		498
SLW F1C	ccggaattcgttagttacttaCAAAGAGGTGGCTTCTTCGGC	for GST fusion construct (782 to 1636) UC = hTRT seq, lc = EcoR I site + 3 stop codons		x	x		499
	SLW F1N / SLW F1C amplify a 893 nt piece of pGRN121 (782 to 1636)						
SLW F2N	cgcggatccgtaactaaaGCCACCTCTTTGGAGGGTGCG	for GST fusion construct (1625 to 2458) UC = hTRT seq, lc = BamH1 site + 2 stop codons		x	x		500
SLW F2C	ccggaattcgttagttacttaAGACCTGAGCAGCTCGACGAC	for GST fusion construct (1625 to 2458) UC = hTRT seq, lc = EcoR I site + 3 stop codons		x	x		501
	SLW F2N / SLW F2C amplify a 872 nt piece of pGRN121 (1625 to 2458)						
SLW F3N	cgcggatccgtaactaaaATGAGTGTGTACGTCGTCGAG	for GST fusion construct (2426 to 3274) UC = hTRT seq, lc = BamH1 site + 2 stop codons		x	x		502
SLW F3C	ccggaattcgttagttacttaGATCCCCTGGCACTGGACG	for GST fusion construct (2426 to 3274) UC = hTRT seq, lc = EcoR I site + 3 stop codons		x	x		503
	SLW F3N / SLW F3C amplify a 887 nt piece of pGRN121 (2426 to 3274)						
SLW F4N	cgcggatccgtaactaaaATCCCGCAGGGCTCCATCCTC	for GST fusion construct (3272 to 4177) UC = hTRT seq, lc = BamH1 site + 2 stop codons		x	x		504
SLW F4C	ccggaattcgttagttacttaGTCCAGGATGGTCTTGAAGTC	for GST fusion construct (3272 to 4177) UC = hTRT seq, lc = EcoR I site + 3 stop codons		x	x		505
	SLW F4N / SLW F4C amplify a 944 nt piece of pGRN121 (3272 to 4177)						
40-60	GGCATCGCGGGGGTGGCCGGG	phosphorothioate			x		506
260-280	GGACACCTGGCGGAAGGAGGG	phosphorothioate			x		507
500-520	GCGTGCCAGCAGGTGAACCAG	phosphorothioate			x		508
770-790	CTCAGGGGCAGCGCCACGCCT	phosphorothioate			x		509

885-905	AGGTGGCTTCTTCGGCGGGTC	phosphorothioate		x	510
1000-1020	GGACAAGGCGTGTCCCAGGGA	phosphorothioate		x	511
1300-1320	GCTGGGGTGACCGCAGCTCGC	phosphorothioate		x	512
1520-1540	GATGAACTTCTTGGTGTTCCT	phosphorothioate		x	513
2110-2130	GTGCGCCAGGCCCTGTGGATA	phosphorothioate		x	514
2295-2315	GCCCATGGGCGGCCTTCTGGA	phosphorothioate		x	515
2450-2470	GAGGCCACTGCTGGCCTCATT	phosphorothioate		x	516
2670-2690	GGGTGAGGTGAGGTGTCACCA	phosphorothioate		x	517
3080-3110	GCTGCAGCACACATGCGTGAAACCTGTACGC	phosphorothioate		x	518
3140-3160	GACGCGCAGGAAAAATGTGGG	phosphorothioate		x	519
3690-3710	CCGAGCGCCAGCCTGTGGGGA	phosphorothioate		x	520
55-75	CAGCGGGGAGCGCGCGGCATC	phosphorothioate		x	521
151-171	CAGCACCTCGCGGTAGTGGCT	phosphorothioate		x	522
TP1.1	TCAAGCCAAACCTGAATCTGAG		x		523
TP1.2	CCCGAGTGAATCTTTCTACGC		x		524
TP1.3	GTCTCTGGCAGTTTCCTCATCCC		x		525
TP1.4	TTTAGGCATCCTCCCAAGCACA		x		526

Please replace the paragraph beginning at page 47, line 15, with the following rewritten paragraph:

In one embodiment, the hTRT protein of the invention is a polypeptide having a sequence as set forth in Figure 17 (SEQ ID NO:2), or a fragment thereof. In another embodiment, the hTRT polypeptide differs from SEQ ID NO:2 by internal deletions, insertions, or conservative substitutions of amino acid residues. In a related embodiment, the invention provides hTRT polypeptides with substantial similarity to SEQ ID NO:2. The invention further provides hTRT polypeptides that are modified, relative to the amino acid sequence of SEQ ID NO:2, in some manner, e.g., truncated, mutated, derivatized, or fused to other sequences (e.g., to form a fusion protein). Moreover, the present invention provides telomerase RNPs comprising an hTRT protein of the invention complexed with a template RNA (e.g., hTR). In other embodiments, one or more telomerase-associated proteins is associated with hTRT protein and/or hTR.

Please replace the paragraph beginning at page 47, line 27, with the following rewritten paragraph:

The invention also provides other naturally occurring hTRT species or nonnaturally occurring variants, such as proteins having the sequence of, or substantial similarity to SEQ ID NO:5 [[Figure 20], SEQ ID NO:10 [Figure 19], and fragments, variants, or derivatives thereof.

Please replace the paragraph beginning at page 47, line 31, with the following rewritten paragraph:

The invention provides still other hTRT species and variants. One example of an hTRT variant may result from ribosome frameshifting of mRNA encoded by the clone 712562 (SEQ ID NO:3 [Figure 18]) or the pro90 variant hTRT shown in SEQ ID NO:4 [Figure 20] and so result in the synthesis of hTRT polypeptides containing all the TRT motifs (for a general example, see, e.g., Tsuchihashi et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:2516; Craigengen et al., 1987, *Cell* 50:1; Weiss, 1990, *Cell* 62:117). Ribosome frameshifting can occur when specific mRNA sequences or secondary structures cause the ribosome to Astall≅ and jump one nucleotide

forwards or back in the sequence. Thus, a ribosome frameshift event on the 712562 mRNA could cause the synthesis of an approximately 523 amino acid residue polypeptide. A ribosome frameshift event on the pro90 sequence could result in a protein with approximately 1071 residues. It will be appreciated that proteins resulting from ribosome frameshifting can also be expressed by synthetic or recombinant techniques provided by the invention.

Please replace the table beginning at page 50, line 22, with the following rewritten table:

NONPROCESSIVE ACTIVITY

```
i)    ---------TTAGGGttag (DNA)          SEQ ID NO:527
          3'-----AUCCCAAUC-----5'   (RNA)

ii)   ---------TTAGggttag  (DNA)         SEQ ID NO:527
          3'-----AUCCCAAUC-------5'   (RNA)

In DNA, University of California, = primer, lc = added nucleotides
```

Please replace the paragraph beginning at page 51, line 3, with the following rewritten paragraph:

A fully non-processive reaction produces only one band in a conventional assay using a single synthetic primer. Because this result could also be produced by other enzymes, such as a terminal transferase activity, it may be desirable in some applications to verify that the product is a result of a telomerase catalytic activity. A telomerase (comprising hTRT) generated band can be distinguished by several additional characteristics. The number of nucleotides added to the end of the primer should be consistent with the position of the primer 3' end. Thus, a --TTAGGG primer should have 4 nucleotides added and a --TTAG primer should have 6 nucleotides added (see above). In practice, two or more sequence permuted primers can be used which have the same overall length but different 5' and 3' endpoints. As an illustrative example, the non-processive extension of primers 5'-TTAGGGTTAGGGTTAGGG (SEQ ID NO:528) and 5'-GTTAGGGTTAGGGTTAGG (SEQ ID NO:529) will generate products whose absolute length will be one nucleotide different (4 added to 5'-TTAGGGTTAGGGTTAGGG (SEQ ID

NO:528) for a 22 nt total length, and 5 added to 5'-GTTAGGGTTAGGGTTAGG (SEQ ID NO:529) for a 23 nt total length). The nucleotide dependence of the reaction should be consistent with the position of the primer terminus. Thus, a --TTAGGG primer product should require dGTP, TTP, and dATP, but not dCTP, and a ---AGGGTT primer product should require dGTP and dATP, but not TTP or dCTP. The activity should be sensitive to RNAase or micrococcal nuclease pre-treatment (see Morin, 1989, *Cell* 59: 521) under conditions that will degrade hTR and so eliminate the template.

Please replace the paragraph beginning at page 53, line 29, with the following rewritten paragraph:

In an alternative embodiment, the hTRT protein is expressed in a cell (e.g., a telomerase negative cell in which hTR is expressed) as a fusion protein (see *infra*) having a label or an "epitope tag" to aid in purification. In one embodiment, the RNP is recovered from the cell using an antibody that specifically recognizes the tag. Preferred tags are typically short or small and may include a cleavage site or other property that allows the tag to be removed from the hTRT polypeptide. Examples of suitable tags include the Xpress™ epitope (Invitrogen, Inc., San Diego CA), and other moieties that can be specifically bound by an antibody or nucleic acid or other equivalent method such as those described in Example 6. Alternative tags include those encoded by sequences inserted, e.g., into SEQ ID NO:1 upstream of the ATG codon that initiates translation of the protein of SEQ ID NO:2, which may include insertion of a (new) methionine initiation codon into the upstream sequence.

Please replace the paragraph beginning at page 55, line 2, with the following rewritten paragraph:

Because hTRT specifically associates with hTR, it can be appreciated that the DNA primer/RNA template for a conventional RT assay can be modified to have characteristics related to hTR and/or a telomeric DNA primer. For example, the RNA can have the sequence $(CCCTAA)_n$, where n is at least 1, or at least 3, or at least 10 or more (SEQ ID NO:530). In one

embodiment, the $(CCCTAA)_n$ region is at or near the 5' terminus of the RNA (similar to the 5' locations of template regions in telomerase RNAs). Similarly, the DNA primer may have a 3' terminus that contains portions of the TTAGGG telomere sequence, for example X_nTTAG (SEQ ID NO:531), X_nAGGG (SEQ ID NO:532), $X_n(TTAGGG)_qTTAG$ (SEQ ID NOS:533-536), etc., where X is a non-telomeric sequence and n is 8-20, or 6-30, and q is 1-4. In another embodiment, the DNA primer has a 5' terminus that is non-complementary to the RNA template, such that when the primer is annealed to the RNA, the 5' terminus of the primer remains unbound. Additional modifications of standard reverse transcription assays that may be applied to the methods of the invention are known in the art.

Please replace the paragraph beginning at page 55, line 16, with the following rewritten paragraph:

Telomerase nucleolytic activity is described in e.g., Morin, 1997, *supra*; Collins and Grieder, *1993, Genes and Development* 7:1364. Telomerase possesses a nucleolytic activity (Joyce and Steitz, 1987, *Trends Biochem. Sci.* 12:288); however, telomerase activity has defining characteristics. Telomerase preferentially removes nucleotides, usually only one, from the 3' end of an oligonucleotide when the 3' end of the DNA is positioned at the 5' boundary of the DNA template sequence, in humans and *Tetrahymena*, this nucleotide is the first G of the telomeric repeat (TTAGG in humans). Telomerase preferentially removes G residues but has nucleolytic activity against other nucleotides. This activity can be monitored. Two different methods are described here for illustrative purposes. One method involves a conventional telomerase reaction with a primer that binds the entire template sequence (i.e., terminating at the template boundary; 5'-TAGGGATTAG (SEQ ID NO:537) in humans). Nucleolytic activity is observed by monitoring the replacement of the last dG residue with a radiolabeled dGTP provided in the assay. The replacement is monitored by the appearance of a band at the size of the starting primer as shown by gel electrophoresis and autoradiography.

Please replace the paragraph beginning at page 56, line 1, with the following rewritten paragraph:

A preferred method uses a DNA primer that has a "blocked" 3' terminus that cannot be extended by telomerase. The 3'-blocked primer can be used in a standard telomerase assay but will not be extended unless the 3' nucleotide is removed by the nucleolytic activity of telomerase. The advantage of this method is that telomerase activity can be monitored by any of several standard means, and the signal is strong and easy to quantify. The blocking of the 3' terminus of the primer can be accomplished in several ways. One method is the addition of a 3'-deoxy-dNTP residue at the 3' terminus of the primer using standard oligonucleotide synthesis techniques. This terminus has a 2' OH but not the 3' OH required for telomerase. Other means of blocking the 3' terminus exist, for instance, a 3' dideoxy terminus, a 3'-amine terminus, and others. An example of a primer for an hTRT nucleolytic assay is 5' -TTAGGGTTAGGGTTA ($G_{3'H}$) (SEQ ID NO:538) where the last residue denotes a 3'-deoxy-guanosine residue (Glen Research, Sterling, VA). Numerous other variations for a suitable primer based on the disclosure are known to those of skill in the art.

Please replace the paragraph beginning at page 57, line 6, with the following rewritten paragraph:

The DNA primer can be any DNA with an affinity for telomerase, such as, for example, a telomeric DNA primer like $(TTAGGG)_n$, where n could be 1-10 and is typically 3-5 (SEQ ID NO:539). The 3' and 5' termini can end in any location of the repeat sequence. The primer can also have 5' or 3' extensions of non-telomeric DNA that could facilitate labeling or detection. The primer can also be derivatized, e.g., to facilitate detection or isolation.

Please replace the paragraph beginning at page 57, line 31, with the following rewritten paragraph:

The present invention, as noted *supra*, provides in addition to recombinant hTRT with a full complement (as described *supra*) of activities, hTRT polypeptides having less than the full

complement of the telomerase activities of naturally occurring telomerase or hTRT or other TRT proteins. It will be appreciated that, in view of the disclosure herein of the RT and telomerase-specific motifs of TRT, alteration or mutation of conserved amino acid residues, such as are found in the motif sequences discussed *supra*, will result in loss-of activity mutants useful for therapeutic, drug screening and characterization, and other uses. For example, as described in Example 1, deletion of motifs B through D in the RT domains of the endogenous TRT gene in *S. pombe* resulted in haploid cells in which telomere progressively shortened to the point where hybridization of a telomere probe to telomeric repeats became almost undetectable, indicating a loss of telomerase catalytic activity. Similarly, alterations in the WxGxS (SEQ ID NO:540) site of motif E can affect telomerase DNA primer binding or function. Additionally, alterations of the amino acids in the motifs A, B', and C can affect the catalytic activity of telomerase. Mutation of the Defendant motif of hTRT can significantly reduce or abolish telomerase activity (see Example 16).

Please replace line 12, page 71 with the following rewritten line:
E. coli (all genes) (SEQ ID NO:638)

Please replace line 32, page 72 with the following rewritten line:
Enteric Bacteria (High Expressing Genes) (SEQ ID NO:639)

Please replace line 49, page 73 with the following rewritten line:
Yeast (All Genes) (SEQ ID NO:640)

Please replace line 6, page 75 with the following rewritten line:
Yeast (High Expressing Genes) (SEQ ID NO:641)

Please replace line 19, page 76 with the following rewritten line:
"Generic" hTRT Protein Encoding Sequence (SEQ ID NO:642)

Please replace line 27, page 78 with the following rewritten line:

Table 10A: Oligonucleotides (SEQ ID NOS:643-721)

Please replace line 41, page 81 with the following rewritten line:

Table 10B (SEQ ID NO:721)

Please replace the paragraph beginning at page 87, line 17, with the following rewritten paragraph:

In a related aspect, the present invention provides antibodies that are specifically immunoreactive with hTRT, including polyclonal and monoclonal antibodies, antibody fragments, single chain antibodies, human and chimeric antibodies, including antibodies or antibody fragments fused to phage coat or cell surface proteins, and others known in the art and described herein. The antibodies of the invention can specifically recognize and bind polypeptides that have an amino acid sequence that is substantially identical to the amino acid sequence set forth in Figure 17 SEQ ID NO:2, or an immunogenic fragment thereof or epitope on the protein defined thereby. The antibodies of the invention can exhibit a specific binding affinity for hTRT of at least about 10^7, 10^8, 10^9, or 10^{10} M^{-1}, and may be polyclonal, monoclonal, recombinant or otherwise produced. The invention also provides anti-hTRT antibodies that recognize an hTRT conformational epitope (e.g., an epitope on the surface of the hTRT protein or a telomerase RNP). Likely conformational epitopes can be identified, if desired, by computer-assisted analysis of the hTRT protein sequence, comparison to the conformation of related reverse transcriptases, such as the p66 subunit of HIV-1 (see, e.g., Figure 3), or empirically. Anti-hTRT antibodies that recognize conformational epitopes have utility, inter alia, in detection and purification of human telomerase and in the diagnosis and treatment of human disease.

Please replace the paragraph beginning at page 88, line 3, with the following rewritten paragraph:

For the production of anti-hTRT antibodies, hosts such as goats, sheep, cows, guinea pigs, rabbits, rats, or mice, may be immunized by injection with hTRT protein or any portion, fragment or oligopeptide thereof which retains immunogenic properties. In selecting hTRT polypeptides for antibody induction, one need not retain biological activity; however, the protein fragment, or oligopeptide must be immunogenic, and preferably antigenic. Immunogenicity can be determined by injecting a polypeptide and adjuvant into an animal (e.g., a rabbit) and assaying for the appearance of antibodies directed against the injected polypeptide (see, e.g., Harlow and Lane, ANTIBODIES: A LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, New York (1988), which is incorporated in its entirety and for all purposes, e.g., at Chapter 5). Peptides used to induce specific antibodies typically have an amino acid sequence consisting of at least five amino acids, preferably at least 8 amino acids, more preferably at least 10 amino acids. Usually they will mimic or have substantial sequence identity to all or a contiguous portion of the amino acid sequence of the protein of SEQ ID NO:2. Short stretches of hTRT protein amino acids may be fused with those of another protein, such as keyhole limpet hemocyanin, and an anti-hTRT antibody produced against the chimeric molecule. Depending on the host species, various adjuvants may be used to increase immunological response.

Please replace the paragraph beginning at page 102, line 19, with the following rewritten paragraph:

In one aspect of the invention, a telomerase modulatory polypeptide that increases telomerase activity in a cell is provided. In one embodiment, the polypeptide is a catalytically active hTRT polypeptide capable of directing the synthesis (in conjunction with an RNA template such as hTR) of human telomeric DNA. This activity can be measured, as discussed above, e.g., using a telomerase activity assay such as a TRAP assay. In one embodiment, the polypeptide is a full-length hTRT protein, having a sequence of, or substantially identical to, the sequence of 1132 residues of SEQ ID NO:2. In another embodiment, the polypeptide is a variant of the hTRT protein of SEQ ID NO:2, such as a fusion polypeptide, derivatized polypeptide, truncated polypeptide, conservatively substituted polypeptide, activity-modified polypeptide, or

the like. A fusion or derivatized protein may include a targeting moiety that increases the ability of the polypeptide to traverse a cell membrane or causes the polypeptide to be delivered to a specified cell type (e.g., liver cells or tumor cells) preferentially or cell compartment (e.g., nuclear compartment) preferentially. Examples of targeting moieties include lipid tails, amino acid sequences such as antennapoedia peptide or a nuclear localization signal (NLS; e.g., *Xenopus* nucleoplasmin Robbins et al., 1991, *Cell* 64:615). Naturally occurring hTRT protein (e.g., having a sequence of, or substantially identical to, SEQ ID NO:2) acts in the cell nucleus. Thus, it is likely that one or more subsequences of SEQ ID NO:2, such as residues 193-196 (PRRR SEQ ID NO:541) and residues 235-240 (PKRPRR SEQ ID NO: 542) act as a nuclear localization signal. The small regions are likely NLSs based on the observation that many NLSs comprise a 4 residue pattern composed of basic amino acids (K or R), or composed of three basic amino acids (K or R) and H or P; a pattern starting with P and followed within 3 residues by a basic segment containing 3 K or R residues out of 4 residues (see, e.g., Nakai et al., 1992, *Genomics* 14:897). Deletion of one or both of these sequences and/or additional localization sequences is expected to interfere with hTRT transport to the nucleus and/or increase hTRT turnover, and is useful for preventing access of telomerase to its nuclear substrates and decreasing proliferative potential. Moreover, a variant hTRT polypeptide lacking NLS may assemble into an RNP that will not be able to maintain telomere length, because the resulting enzyme cannot enter the nucleus.

Please replace the paragraph beginning at page 103, line 17, with the following rewritten paragraph:

The hTRT polypeptides of the invention will typically be associated in the target cell with a telomerase RNA, such as hTR, especially when they are used to increase telomerase activity in a cell. In one embodiment, an introduced hTRT polypeptide associates with an endogenous hTR to form a catalytically active RNP (e.g., an RNP comprising the hTR and a full-length polypeptide having a sequence of SEQ ID NO:2). The RNP so-formed may also associate with other, e.g., telomerase-associated, proteins. In other embodiments, telomerase RNP (containing

hTRT protein, hTR and optionally other components) is introduced as a complex to the target cell.

Please replace the paragraph beginning at page 106, line 7, with the following rewritten paragraph:

In one embodiment, the antisense sequence is complementary to relatively accessible sequences of the hTRT mRNA (e.g., relatively devoid of secondary structure). This can be determined by analyzing predicted RNA secondary structures using, for example, the MFOLD program (Genetics Computer Group, Madison WI) and testing *in vitro* or *in vivo* as is known in the art. Examples of oligonucleotides that may be tested in cells for antisense suppression of hTRT function are those capable of hybridizing to (i.e., substantially complementary to) the following positions from SEQ ID NO:1: 40-60; 260-280; 500-520; 770-790; 885-905; 1000-1020 ; 1300-1320; 1520-1540; 2110-2130; 2295-2315; 2450-2470; 2670-2690; 3080-3110; 3140-3160; and 3690-3710. Another useful method for identifying effective antisense compositions uses combinatorial arrays of oligonucleotides (see, e.g., Milner et al., 1997, *Nature Biotechnology* 15:537).

Please replace the paragraph beginning at page 106, line 23, with the following rewritten paragraph:

The antisense nucleic acids (DNA, RNA, modified, analogues, and the like) can be made using any suitable method for producing a nucleic acid, such as the chemical synthesis and recombinant methods disclosed herein. In one embodiment, for example, antisense RNA molecules of the invention may be prepared by *de novo* chemical synthesis or by cloning. For example, an antisense RNA that hybridizes to hTRT mRNA can be made by inserting (ligating) an hTRT DNA sequence (e.g., SEQ ID NO:1, or fragment thereof) in reverse orientation operably linked to a promoter in a vector (e.g., plasmid). Provided that the promoter and, preferably termination and polyadenylation signals, are properly positioned, the strand of the

inserted sequence corresponding to the noncoding strand will be transcribed and act as an antisense oligonucleotide of the invention.

Please replace the paragraph beginning at page 107, line 2, with the following rewritten paragraph:

The antisense oligonucleotides of the invention can be used to inhibit telomerase activity in cell-free extracts, cells, and animals, including mammals and humans. For example, the phosphorothioate antisense oligonucleotides:

A) 5'-GGCATCGCGGGGGTGGCCGGG SEQ ID NO:506

B) 5'-CAGCGGGGAGCGCGCGGCATC SEQ ID NO:521

C) 5'-CAGCACCTCGCGGTAGTGGCT SEQ ID NO:522

D) 5'-GGACACCTGGCGGAAGGAGGG SEQ ID NO:507

can be used to inhibit telomerase activity. At 10 micromolar concentration each oligonucleotide, mixtures of oligonucleotides A and B; A, B, C, and D; and A, C, and D inhibited telomerase activity in 293 cells when treated once per day for seven days. Inhibition was also observed when an antisense hTR molecule (5'-GCTCTAGAATGAAGGGTG-3'; 3' SEQ ID NO:543) was used in combination with oligonucleotides A, B, and C; A, B, and D; and A and C. Useful control oligonucleotides in such experiments include:

S1) 5'-GCGACGACTGACATTGGCCGG SEQ ID NO:544

S2) 5'-GGCTCGAAGTAGCACCGGTGC SEQ ID NO:545

S3) 5'-GTGGGAACAGGCCGATGTCCC SEQ ID NO:546

Please replace the paragraph beginning at page 107, line 20, with the following rewritten paragraph:

To determine the optimum antisense oligonucleotide of the invention for the particular application of interest, one can perform a scan using antisense oligonucleotide sets of the invention. One illustrative set is the set of 30-mer oligonucleotides that span the hTRT mRNA and are offset one from the next by fifteen nucleotides (i.e., ON1 corresponds to

positions 1-30 and is TCCCACGTGCGCAGCAGGACGCAGCGCTGC (SEQ ID NO:547), ON2 corresponds to positions 16-45 and is GCCGGGGCCAGGGCTTCCCACGTGCGCAGC (SEQ ID NO:548), and ON3 corresponds to positions 31-60 and is GGCATCGCGGGGGTGGCCGGGGCCAGGGCT (SEQ ID NO:549), and so on to the end of the mRNA). Each member of this set can be tested for inhibitory activity as disclosed herein. Those oligonucleotides that show inhibitory activity under the conditions of interest then identify a region of interest, and other oligonucleotides of the invention corresponding to the region of interest (i.e., 8-mers, 10-mers, 15-mers, and so on) can be tested to identify the oligonucleotide with the preferred activity for the application.

Please replace the paragraph beginning at page 108, line 1, with the following rewritten paragraph:

Exemplary antisense oligonucleotides include 5'-GGCATCGCGGGGGTG GCCGGGGCCAGGGCT-3' (SEQ ID NO:722) (corresponding to nucleotide positions 31-60 of hTRT); 5'-GCGCA GCGTGCCAGCAGGTGAACCAGCACG-3' (SEQ ID NO:723) (corresponding to positions 496-525); 5'- GCCCGTTCGCATCCCAGACGCCTTCGGGGT-3' (SEQ ID NO:724) (corresponding to positions 631-660); and 5'-ACGCTATGGTTCCAGGCCCGTTCGCATCCC-3' (SEQ ID NO:725) (corresponding to positions 646-675). When ACHN cells (NCI #503755) or 293 cells were treated for three days with 10 μM of phosphorothioate oligonucleotides with any of the four sequences *supra*, inhibition of telomerase activity by about 50%-90% (compared to control untreated cells) as measured by a TRAP assay, was observed.

Please replace the paragraph beginning at page 112, line 9, with the following rewritten paragraph:

As noted, the present invention also provides methods and reagents for gene replacement therapy (i.e., replacement by homologous recombination of an endogenous hTRT gene with a recombinant gene). Vectors specifically designed for integration by homologous

recombination may be used. Important factors for optimizing homologous recombination include the degree of sequence identity and length of homology to chromosomal sequences. The specific sequence mediating homologous recombination is also important, because integration occurs much more easily in transcriptionally active DNA. Methods and materials for constructing homologous targeting constructs are described by e.g., Mansour et al., 1988, *Nature* 336: 348; Bradley et al., 1992, *Bio/Technology* 10: 534. See also, U.S. Patent Nos. 5,627,059; 5,487,992; 5,631,153; and 5,464,764. In one embodiment, gene replacement therapy involves altering or replacing all or a portion of the regulatory sequences controlling expression of the hTRT gene that is to be regulated. For example, the hTRT promoter sequences (e.g., such as are found in SEQ ID NO:6) may be disrupted (to decrease hTRT expression or to abolish a transcriptional control site) or an exogenous promoter (e.g., to increase hTRT expression) substituted.

Please replace the paragraph beginning at page 120, line 1, with the following rewritten paragraph:

In one embodiment, a polynucleotide comprising a sequence encoding a polypeptide of SEQ ID NO:2, which sequence is operably linked to a promoter (e.g., a constitutively expressed promoter, e.g., a sequence of SEQ ID NO:6, is introduced into the cell. In one embodiment the polynucleotide comprises a sequence of SEQ ID NO:1. Preferably the polynucleotide includes polyadenylation and termination signals. In other embodiments, additional elements such as enhancers or others discussed *supra* are included. In an alternative embodiment, the polynucleotide does not include a promoter sequence, such sequence being provided by the target cell endogenous genome following integration (e.g., recombination, e.g., homologous recombination) of the introduced polynucleotide. The polynucleotide may be introduced into the target cell by any method, including any method disclosed herein, such as lipofection, electroporation, virosomes, liposomes, immunoliposomes, polycation:nucleic acid conjugates, naked DNA).

Please replace the paragraph beginning at page 147, line 17, with the following rewritten paragraph:

One example of an hTRT variant gene product that may be detected is an hTRT RNA such as a product (SEQ ID NO:4) described *supra* and in Example 9. The biological function, if any, of the Δ182 variant(s) is not known; however, the truncated hTRT protein putatively encoded by the variant may be involved in regulation of telomerase activity, e.g., by assembling a non-functional telomerase RNP that titrates telomerase components. Alternatively, negative regulation of telomerase activity could be accomplished by directing hTRT pre-mRNA (nascent mRNA) processing in a manner leading to elimination of the full length mRNA and reducing hTRT mRNA levels and increasing Δ182 hTRT RNA levels. For these and other reasons, the ability to detect Δ182 variants is useful. In addition, it will sometimes be desirable, in samples in which two species of hTRT RNA are present (such as a Δ182 hTRT RNA and hTRT RNA encoding the full-length hTRT protein) to compare their relative and/or absolute abundance.

Please replace the paragraph beginning at page 147, line 29, with the following rewritten paragraph:

The invention provides a variety of methods for detection of Δ182 variants. For example, amplification using primer pairs spanning the 182 basepair deletion will result in different sized products corresponding to the deleted and undeleted hTRT RNAs, if both are present, which can be distinguished on the basis of size (e.g., by gel electrophoresis). Examples of primer pairs useful for amplifying the region spanning the 182 bp deletion include TCP1.14 and TCP1.15 (primer set 1), or TCP1.25 and billTCP6 (primer set 2) (see Table 2). These primer pairs can be used individually or in a nested PCR experiment where primer set 1 is used first. It will also be apparent to one of skill that hybridization methods (e.g., Northern hybridization) or RNAse protection assays

using an hTRT nucleic acid probe of the invention can be used to detect and distinguish hTRT RNA variants.

Please replace the paragraph beginning at page 148, line 8, with the following rewritten paragraph:

Another suitable method entails PCR amplification (or the equivalent) using three primers. Analogous to the semi-competitive quantitative PCR method described in greater detail supra, one primer is specific to each of the hTRT RNA species (e.g., as illustrated in Table 4) and one primer is complementary to both species (e.g., TCP1.25 (2270-2288)). An example of a primer specific to SEQ ID NO:1 is one that anneals within the 182 nucleotide sequence (i.e., nucleotides 2345 to 2526 of SEQ ID NO:1), e.g., TCP1.73 (2465-2445). For example, a primer specific to SEQ ID NO:4 (a Δ182 variant) is one that anneals at nucleotides 2358 to 2339 of SEQ ID NO:4 (i.e., the site corresponding to the 182 nucleotide insertion in SEQ ID NO:1). The absolute abundance of the Δ182 hTRT mRNA species or its relative abundance compared to the species encoding the full-length hTRT protein can be analyzed for correlation to cell state (e.g., capacity for indefinite proliferation). It will be appreciated that numerous other primers or amplification or detection methods can be selected based on the present disclosure.

Please replace Table 4 beginning at page 148, line 21, with the following rewritten table:

TABLE 4

ILLUSTRATIVE PRIMERS

Δ182 species (e.g., SEQ ID NO:4) specific primer:

5'-GGCACTGGACGTAGGACGTG-3 (SEQ ID NO:550)

hTRT (SEQ ID NO:1) specific primer (TCP1.73):

5'-CACTGCTGGCCTCATTCAGGG-3 z(SEQ ID NO:445)

Common (forward) primer (TCP1.25):

5'-TACTGCGTGCGTCGGTATG-3' (SEQ ID NO:399)

Please replace the paragraph beginning at page 177, line 17, with the following rewritten paragraph:

Altering the expression of endogenous genes by homologous recombination can also be accomplished by using nucleic acid sequences comprising the structural gene in question. Upstream sequences are utilized for targeting heterologous recombination constructs. Utilizing TRT structural gene sequence information, such as SEQ ID NO:1, one of skill in the art can create homologous recombination constructs with only routine experimentation. Homologous recombination to alter expression of endogenous genes is described in U.S. Patent 5,272,071, and WO 91/09955, WO 93/09222, WO 96/29411, WO 95/31560, and WO 91/12650. Homologous recombination in mycobacteria is described by Azad (1996) Proc. Natl. Acad. Sci. USA 93:4787; Baulard (1996) J. Bacteriol.178:3091; and Pelicic (1996) Mol. Microbiol. 20:919. Homologous recombination in animals has been described by Moynahan (1996) Hum. Mol. Genet. 5:875, and in plants by Offringa (1990) EMBO J. 9:3077.

Please replace the paragraph beginning at page 189, line 4, with the following rewritten paragraph:

As used herein, the term "**substantial identity,**" "**substantial sequence identity,**" or "**substantial similarity**" in the context of nucleic acids, refers to a measure of sequence similarity between two polynucleotides. Substantial sequence identity can be determined by hybridization under stringent conditions, by direct comparison, or other means. For example, two polynucleotides can be identified as having substantial sequence identity if they are capable of specifically hybridizing to each other under stringent hybridization conditions. Other degrees of sequence identity (e.g., less than "substantial") can be characterized by hybridization under different conditions of stringency. Alternatively, substantial sequence identity can be described as a percentage identity between two nucleotide (or polypeptide) sequences. Two sequences are considered substantially identical when they are at least about 60% identical, preferably at least about 70% identical, or at least about 80% identical, or at least about 90% identical, or at least about 95% or 98% to 100% identical. Percentage sequence (nucleotide or amino acid) identity is

typically calculated by determining the optimal alignment between two sequences and comparing the two sequences. For example an exogenous transcript used for protein expression can be described as having a certain percentage of identity or similarity compared to a reference sequence (e.g., the corresponding endogenous sequence). Optimal alignment of sequences may be conducted using the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48: 443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection. The best alignment (i.e., resulting in the highest percentage of identity) generated by the various methods is selected. Typically these algorithms compare the two sequences over a "comparison window" (usually at least 18 nucleotides in length) to identify and compare local regions of sequence similarity, thus allowing for small additions or deletions (i.e., gaps). Additions and deletions are typically 20 percent or less of the length of the sequence relative to the reference sequence, which does not comprise additions or deletions. It is sometimes desirable to describe sequence identity between two sequences in reference to a particular length or region (e.g., two sequences may be described as having at least 95% identity over a length of at least 500 basepairs). Usually the length will be at least about 50, 100, 200, 300, 400 or 500 basepairs, amino acids, or other residues. The percentage of sequence identity is calculated by comparing two optimally aligned sequences over the region of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, or U) occurs in both sequences to yield the number of matched positions, and determining the number (or percentage) of matched positions as compared to the total number of bases in the reference sequence or region of comparison. An additional algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul (1990) *J. Mol. Biol.* 215: 403-410; and Shpaer (1996) *Genomics* 38:179-191. Software for performing BLAST analyses is publicly available at the National Center for Biotechnology

Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, *supra*.). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The term BLAST refers to the BLAST algorithm which performs a statistical analysis of the similarity between two sequences; see, e.g., Karlin (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid can be considered similar to a TRT nucleic acid if the smallest sum probability in a comparison of the test nucleic acid to an TRT nucleic acid is less than about 0.5, 0.2, 0.1, 0.01, or 0.001. Alternatively, another indication that two nucleic acid sequences are similar is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid. It will be recognized that homologous non-human TRT polynucleotides may have less that "substantial" nucleotide identity in certain regions, as the term "substantial identity" is defined herein. For example, *Euplotes* TRT is substantially less than about 60%

identical to the hTRT polynucleotide of SEQ ID NO:1 in certain regions, although the two genes are homologs.

Please replace the paragraph beginning at page 191, line 13, with the following rewritten paragraph:

As used herein, the terms "**substantial identity**," "**substantial sequence identity**,"or "**substantial similarity**" in the context of a polypeptide, refers to a degree of similarity between two polypeptides in which a polypeptides comprises a sequence with at least 70% sequence identity to a reference sequence, or 80%, or 85% or up to 100% sequence identity to the reference sequence, or most preferably 90% identity over a comparison window of about 10-20 amino acid residues. Amino acid sequence similarity, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. See Needleham et al. (1970) J. Mol. Biol. 48: 443-453; and Sankoff et al., 1983, *Time Warps, String Edits, and Macromolecules, The Theory and Practice of Sequence Comparison*, Chapter One, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA, and the University of Wisconsin Genetics Computer Group, Madison, WI. As will be apparent to one of skill, the terms "substantial identity", "substantial similarity" and "substantial sequence identity" can be used interchangeably with regard to polypeptides or polynucleotides. It will be recognized that homologous non-human TRT polypeptides may have less that "substantial" sequence identity in certain regions, as the term "substantial identity" is defined herein. For example, *Euplotes* TRT protein is substantially less than about 60% identical to the hTRT polynucleotide of SEQ ID NO:2 in certain regions, although the two genes are homologs. In the context of TRT polypeptides from different species, for example, "significant homology" at the amino acid sequence means at least about 20% sequence identity in region of about 20 to about 40 residues, or at least about 40% sequence identity in region of at least about 20% sequence identity.

Please replace the paragraph beginning at page 196, line 16, with the following rewritten paragraph:

To isolate a full length cDNA clone, a cDNA library derived form the human 293 cell line (described above) which expresses high levels of telomerase activity, was screened. A lambda cDNA library from the 293 cell line was partitioned into 25 pools containing about 200,000 plaques each. Each pool was screened by PCR with the primer pair 5'-CGGAAGAGTGTCTGGAGCAA-3' (SEQ ID NO:551) and 5'-GGATGAAGCGGAGTCTGGA-3' (SEQ ID NO:459). Six subpools of one positive primary pool were further screened by PCR using this same primer pair. For both the primary and the secondary subpool screening, hTRT was amplified for a total of 31 cycles at: 94^{O}C, 45 seconds; 60^{O}C, 45 seconds; and 72^{O}C, 90 seconds. As a control, RNA of the house-keeping enzyme GAPDH was amplified using the primer pair 5'-CTCAGACACCATGGGGAAGGTGA-3' (SEQ ID NO:552) and 5'-ATGATCTTGAGGCTGTTGTCATA-3' (SEQ ID NO:553) for a total of 16 cycles at 94^{O}C, 45 seconds; 55^{O}C, 45 seconds; and 72^{O}C, 90 seconds.

Please replace the paragraph beginning at page 199, line 11, with the following rewritten paragraph:

To this preparation, 1.5 nmol of each of two competitor DNA oligonucleotides (5'-TAGACCTGTTAGTGTACATTTGAATTGAAGC-3'; SEQ ID NO:554 and 5'-TAGACCTGTTAGGTTGGATTTGTGGCATCA-3'; SEQ ID NO:552), 50 μg yeast RNA (Sigma), and 0.3 nmol of biotin-labeled telomerase-specific oligonucleotide (5'-biotin-TAGACCTGTTA-(rmeG)$_2$-(rmeU)$_4$-(rmeG)$_4$-(rmeU)$_4$-rmeG-3'; SEQ ID NO:556), were added per ml of the pool. The 2-O-methyribonucleotides of the telomerase specific oligonucleotides were complem entary to the telomerase RNA; template region; the deoxyribonucleotides were not complementary. The inclusion of competitor, non-specific DNA oligonucleotides increased the efficiency of the purification, as the effects of nucleic acid binding proteins and other components in the mixture that would either bind to the affinity oligonucleotide or remove the telomerase from the mixture were minimized.

Please replace the paragraph beginning at page 200, line 9, with the following rewritten paragraph:

Telomerase was eluted from the column material by adding 1 nmol of displacement deoxyoligonucleotide (5'-$CA_4C_4A_4C_2TA_2CAG_2TCTA$-3'; SEQ ID NO:557), per ml of column material and incubating at 25°C for 30 minutes. The material was centrifuged for 2 minutes at 14,000 rpm in a microcentrifuge (Eppendorf), and the eluate collected. The elution procedure was repeated twice more, using fresh displacement oligonucleotide each time. As mentioned above, because the displacement oligonucleotide was complementary to the affinity oligonucleotide, it formed a more thermodynamically stable complex with the affinity oligonucleotide than P-40. Thus, addition of the displacement oligonucleotide to an affinity-bound telomerase resulted in efficient elution of telomerase under native conditions. The telomerase appeared to be approximately 50% pure at this stage, as judged by analysis on a protein gel. The affinity purification of telomerase and elution with a displacement oligonucleotide is shown in Figure 26 (panels A and B, respectively). In this Figure, the 2'-*O*-methyl sugars of the affinity oligonucleotide are indicated by the bold line. The black and shaded oval shapes in this Figure are intended to represent graphically the protein subunits of the present invention.

Please replace the paragraph beginning at page 203, line 2, with the following rewritten paragraph:

A construct for phage T7 RNA polymerase transcription of *E. aediculatus* telomerase RNA was produced, using (PCR). The telomerase RNA gene was amplified with primers that annealed to either end of the gene. The primer that annealed at the 5' end also encoded a hammerhead ribozyme sequence to generate the natural 5' end upon cleavage of the transcribed RNA, a T7-promoter sequence, and an *Eco*RI site for subcloning. The sequence of this 5' primer was 5'-GCGGGAATTCTAA TACGACTCACTATAGGGAAGAAACTCTGATGAGGCCGAAAGGCCGAAACTCCACGA AAGTGGAGTAAGTTTCTCGATAATTGATCTGTAG-3' (SEQ ID NO:558). The 3' primer

included an *Ear*I site for termination of transcription at the natural 3' end, and a *Bam*HI site for cloning. The sequence of this 3' primer was 5'-CGGGGATCCTCTTCAAAAG ATGAGAGGACAGCAAAC-3' (SEQ ID NO:559). The PCR amplification product was cleaved with *Eco*RI and *Bam*HI, and subcloned into the respective sites of pUC19 (NEB), to give "pEaT7." The correctness of this insert was confirmed by DNA sequencing. T7 transcription was performed as described by Zaug et al., Biochemistry 33:14935 [1994], with *Ear*I-linearized plasmid. RNA was gel-purified and the concentration was determined (an A_{260} of 1 = 40 μg/ml). This RNA was used as a standard to determine the telomerase RNA present in various preparations of telomerase.

Please replace Table 6, beginning at page 207, line 16, with the following rewritten table:

TABLE 6. Primer Sequences

Lane	Primer Sequence (5' to 3')	SEQ ID NO.:
1-3	$C_4(A_4C_4)_3CACA(G_4T_4)_3G_4$	560
4-6	$C_2(A_4C_4)_3CACA(G_4T_4)_3G_4$	561
7-9	$(A_4C_4)_3CACA(G_4T_4)_3G_4$	562
10-12	$A_2C_4(A_4C_4)_2CACA(G_4T_4)_3G_4$	563
13-15	$C_4(A_4C_4)_2CACA(G_4T_4)_3$	564
16-18	$(A_4C_4)_3CACA(G_4T_4)_3$	565
19-21	$A_2C_4(A_4C_4)_2CACA(G_4T_4)_3$	566
22-24	$C_4(A_4C_4)_2CACA(G_4T_4)_3$	564
25-27	$C_2(A_4C_4)_2CACA(G_4T_4)_3$	567
28-30	$(A_4C_4)_2CACA(G_4T_4)_3$	568

Please replace the paragraph beginning at page 208, line 22, with the following rewritten paragraph:

As shown in Figure 31, double-stranded, blunt-ended oligonucleotides were not substrates for telomerase. To determine whether these molecules would bind to telomerase, a competition experiment was performed. In this experiment, 2 nM of 5'-end labeled substrate with the sequence $(G_4T_4)_2$ (SEQ ID NO:114), or a hairpin substrate with a six base overhang were extended with 0.125 nM telomerase (Figure 31, lanes 25-27). Although the same unlabeled oligonucleotide substrates competed efficiently with labeled substrate for extension, no reduction of activity was observed when the double-stranded blunt-ended hairpin oligonucleotides were used as competitors, even in the presence of 100-fold excess hairpins.

Please replace the paragraph beginning at page 209, line 6, with the following rewritten paragraph:

In this Example, the cloning of the 123 kDa polypeptide of Euplotes telomerase (*i.e.,* the 123 kDa protein subunit) is described. In this study, an internal fragment of the telomerase gene was amplified by PCR, with oligonucleotide primers designed to match peptide sequences that were obtained from the purified polypeptide obtained in Part D, above. The polypeptide sequence was determined using the nanoES tandem mass spectroscopy methods known in the art and described by Calvio *et al.,* RNA 1:724-733 [1995]. The oligonucleotide primers used in this Example had the following sequences, with positions that were degenerate shown in parentheses--5'-TCT(G/A)
AA(G/A)TA(G/A)TG(T/G/A)GT(G/A/T/C)A(T/G/A)(G/A)TT(G/A)TTCAT-3' (SEQ ID NO:569), and
5'-GCGGATCCATGAA(T/C)CC(A/T)GA(G/A)AA(T/C)CC(A/T)AA(T/C)GT-3' (SEQ ID NO:570).

Please replace the paragraph beginning at page 210, line 5, with the following rewritten paragraph:

In this Example, the cloning of the 43 kDa polypeptide of telomerase (*i.e.*, the 43 kDa protein subunit) is described. In this study, an internal fragment of the corresponding telomerase gene was amplified by PCR, with oligonucleotide primers designed to match peptide sequences that were obtained from the purified polypeptide obtained in Part D, above. The polypeptide sequence was determined using the nanoES tandem mass spectroscopy methods known in the art and described by Calvio *et al., supra*. The oligonucleotide primers used in this Example had the following sequences--5'-NNNGTNAC(C/T/A)GG(C/T/A)AT(C/T/A)AA(C/T)AA-3' (SEQ ID NO:571), and 5'-(T/G/A)GC
(T/G/A)GT(C/T)TC(T/C)TG(G/A)TC(G/A)TT(G/A)TA-3' (SEQ ID NO:572). In this sequence, "N" indicates the presence of any of the four nucleotides (*i.e.*, A, T, G, or C).

Please replace the paragraph beginning at page 210, line 24, with the following rewritten paragraph:

Further downstream, the protein sequence appears to be encoded by different reading frames, as none of the three frames is uninterrupted by stop codons. Furthermore, peptide sequences from purified protein are encoded in all three frames. Therefore, this gene appears to contain intervening sequences, or in the alternative, the RNA is edited. Other possibilities include ribosomal frame-shifting or sequence errors. However, the homology to the La-protein sequence remains of significant interest. Again, in *Euplotes*, the "UGA" codon encodes a cysteine residue.

Please replace the paragraph beginning at page 213, line 21, with the following rewritten paragraph:

The oligonucleotide sequences used as the primers were as follows:
5'-(T/C)A(A/G)AC(T/A/C)AA(G/A)GG(T/A/C)AT(T/C)CC(C/T/A)(C/T)A(G/A)

GG-3' (SEQ ID NO:573) and 5'-(G/A/T)GT(G/A/T)ATNA(G/A)NA(G/A)(G/A)TA(G/A)TC(G/A)TC-3' (SEQ ID NO:574). Positions that were degenerate are shown in parentheses, with the alternative bases shown within the parenthesis. "N" represents any of the four nucleotides.

Please replace the paragraph beginning at page 214, line 30, with the following rewritten paragraph:

A first PCR run was conducted using *Tetrahymena* template macronuclear DNA isolated using methods known in the art, and the 24-mer forward primer with the sequence 5' biotin-GCCTATTT(TC)TT(TC)TA(TC)(GATC)(GATC) (GATC)AC(GATC)GA-3' (SEQ ID NO:575) designated as "K231," corresponding to the FFYXTE SEQ ID NO:360 region, and the 23-mer reverse primer with the sequence 5'-CCAGATAT(GATC)A (TGA)(GATC)A(AG)(AG)AA(AG)TC(AG)TC- 3' (SEQ ID NO:576), designated as "K220," corresponding to the DDFL(FIL)I (SEQ ID NO:577) region. This PCR reaction contained 2.5 µl DNA (50 ng), 4 µl of each primer (20 µM), 3 µl 10x PCR buffer, 3 µl 10x dNTPs, 2 µl Mg, 0.3 µl *Taq*, and 11.2 µl dH_2O. The mixture was cycled for 8 cycles of 94°C for 45 seconds, 37°C for 45 seconds, and 72 °C for 1 minute.

Please replace the paragraph beginning at page 215, line 17, with the following rewritten paragraph:

A second PCR run was conducted using Tetrahymena macronuclear DNA template isolated using methods known in the art, and the 23-mer forward primer with the sequence 5'-ACAATG(CA)G(GATC)(TCA)T(GATC)(TCA)T(GATC)CC (GATC)AA(AG)AA-3' (SEQ ID NO:578), designated as "K228," corresponding to the region R(LI)(LI)PKK (SEQ ID NO:579), and a reverse primer with the sequence 5'-ACGAATC(GT)(GATC)G (TAG)AT(GATC)(GC)(TA)(AG)TC(AG)TA(AG)CA 3' (SEQ ID NO:580), designated "K224," corresponding to the CYDSIPR (SEQ ID NO:581) region. This PCR reaction contained 2.5 µl DNA (50 ng), 4 µl of each primer (20 µM), 3 µl 10x PCR buffer, 3 µl 10x dNTPs, 2 µl Mg, 0.3

μl α $-^{32}$P dATP, 0.3 μl Taq, and 10.9 μl dH_2O. This reaction was run on a 5% denaturing polyacrylamide gel, and the appropriate region was cut out of the gel. These products were reamplified for an additional 34 cycles, under the conditions listed above, with the exception being that a 42°C annealing temperature was used.

Please replace the paragraph beginning at page 216, line 5, with the following rewritten paragraph:

To isolate the *Tetrahymena* band, the round 1 eluate was reamplified with the forward primer K228 and reverse primer K227 with the sequence 5'- CAATTCTC(AG)TA(AG)CA(GATC)(CG)(TA)(CT)TT(AGT)AT(GA)TC-3' (SEQ ID NO:582), corresponding to the DIKSCYD (SEQ ID NO:583) region. The PCR reactions were conducted as described above. The reaction products were run on a 5% polyacrylamide gel; the band corresponding to approximately 295 nucleotides was cut from the gel and sequenced.

Please replace the paragraph beginning at page 216, line 11, with the following rewritten paragraph:

The clone designated as 168-3 was sequenced. The DNA sequence (including the primer sequences) was found to be:
GATTACTCCCGAAGAAAGGATCTTTCCGTCCAATCATGACTTTCTTAAGAAAGGACA
AGCAAAAAAATATTAAGTTAAATCTAAATTAAATTCTAATGGATAGCCAACTTGTGT
TTAGGAATTTAAAAGACATGCTGGGATAAAAGATAGGATACTCAGTCTTTGATAATA
AACAAATTTCAGAAAAATTTGCCTAATTCATAGAGAAATGGAAAAATAAAGGAAGA
CCTCAGCTATATTATGTCACTCTAGACATAAAGACTTGCTAC (SEQ ID NO:584).

Please replace the paragraph beginning at page 216, line 18, with the following rewritten paragraph:

Additional sequence of this gene was obtained by PCR using one unique primer designed to match the sequence from 168-3 ("K297" with the sequence 5'-GAGTGACATAATATACGTGA-3' (SEQ ID NO:585); and the K231 (FFYXTE; SEQ ID

NO:360) primer. The sequence of the fragment obtained from this reaction, together with 168-3 is as follows (without the primer sequences):

AAACACAAGGAAGGAAGTCAAATATTCTATTACCGTAAACCAATATGGAAATTAGT
GAGTAAATTAACTATTGTCAAAGTAAGAATTTAGTTTTCTGAAAAGAATAAATAAAT
GAAAAATAATTTTTATCAAAAAATTTAGCTTGAAGAGGAGAATTTGGAAAAAGTTG
AAGAAAAATTGATACCAGAAGATTCATTTTAGAAATACCCTCAAGGAAAGCTAAGG
ATTATACCTAAAAAAGGATCTTTCCGTCCAATCATGACTTTCTTAAGAAAGGACAAG
CAAAAAAATATTAAGTTAAATCTAAATTAAATTCTAATGGATAGCCAACTTGTGTTT
AGGAATTTAAAAGACATGCTGGGATAAAAGATAGGATACTCAGTCTTTGATAATAA
ACAAATTTCAGAAAAATTTGCCTAATTCATAGAGAAATGGAAAAATAAAGGAAGAC
CTCAGCTATATTATGTCACTCTA (SEQ ID NO:586).

Please replace the paragraph beginning at page 217, line 1, with the following rewritten paragraph:

The amino acid sequence corresponding to this DNA fragment was found to be:
KHKEGSQIFYYRKPIWKLVSKLTIVKVRIQFSEKNKQMKNNFYQKIQLEEENLEKVEEKL
IPEDSFQKYPQGKLRIIPKKGSFRPIMTFLRKDKQKNIKLNLNQILMDSQLVFRNLKDML
GQKIGYSVFDNKQISEKFAQFIEKWKNKGRPQLYYVTL (SEQ ID NO:228).

Please replace the paragraph beginning at page 218, line 15, with the following rewritten paragraph:

To obtain additional sequence information, 3' and 5' RT PCR were conducted on the telomerase candidate identified in Figure 58. Figure 59 provides a schematic of the 3' RT PCR strategy used. First, cDNA was prepared from mRNA using the oligonucleotide primer "Q_T," (5'-CCA GTG AGC AGA GTG ACG AGG ACT CGA GCT CAA GCT TTT TTT TTT TTT TT-3'; SEQ ID NO:587), then using this cDNA as a template for PCR with "Q_O" (5'-CCA GTG AGC AGA GTG ACG-3'; SEQ ID NO:588), and a primer designed based on the original degenerated PCR reaction (*i.e.,* "M2-T" with the sequence 5'-G TGT CAT TTC TAT ATG GAA GAT TTG ATT GAT G-3'; SEQ ID NO:589). The second PCR reaction (*i.e.,* nested PCR) with "Q_I" (5'-GAG GAC TCG AGC TCA AGC-3'; SEQ ID NO:590), and another PCR primer designed with sequence derived from the original degenerate PCR reaction or "M2-T2" (5'-AC CTA TCG TTT ACG AAA AAG AAA GGA TCA GTG-3'; SEQ ID NO:591). The buffers used in this PCR were the same as described above, with amplification conducted beginning with a ramp up of 94° for 5 min, followed by 30 cycles of 94° for 30 sec, 55°C for 30 sec, and 72°C for 3 min, followed by 7 minutes at 72°C. The reaction products were stored at 4°C until use.

Please replace the paragraph beginning at page 219, line 16, with the following rewritten paragraph:

As the cDNA version of gene produced to this point was not complete, 5' RT-PCR was conducted to obtain a full length clone. The strategy is schematically shown in Figure 62. In this experiment, cDNA was prepared using DNA oligonucleotide primer "M2-B" (5'-CAC TGA TCC TTT CTT TTT CGT AAA CGA TAG GT-3'; SEQ ID NO:592) and "M2-B2" (5'-C ATC AAT CAA ATC TTC CAT ATA GAA ATG ACA-3'; SEQ ID NO:593), designed from known regions of tez1 identified previously. An oligonucleotide linker PCR Adapt SfiI with a phosphorylated 5' end ("P") (P-GGG CCG TGT TGG CCT AGT TCT CTG CTC-3' SEQ ID NO:594; was then ligated at the 3' end of this cDNA, and this construct was used as the template for nested PCR. In the first round of PCR, PCR Adapt SFI and M2-B were used as the primers; while PCR Adapt SfiI
(5'-GAG GAG GAG AAG AGC AGA GAA CTA GGC CAA CAC GCC CC-3'; SEQ ID

NO:595), and M2-B2 were used as primers in the second round. Nested PCR was used to increase specificity of reaction.

Please replace the paragraph beginning at page 221, line 25, with the following rewritten paragraph:

Sanger dideoxy sequencing and other methods were used, as known in the art to obtain complete sequence information of clone 712562. Some of the primers used in the sequencing are shown in Table 7. These primers were designed to hybridize to the clone, based on sequence complementarity to either plasmid backbone sequence or the sequence of the human cDNA insert in the clone.

Please replace TABLE 7, beginning at page 222, line 2, with the following rewritten table:

Table 7. Primers

Primer	Sequence	SEQ ID NO:
TCP1.1	GTGAAGGCACTGTTCAGCG	377
TCP1.2	GTGGATGATTTCTTGTTGG	381
TCP1.3	ATGCTCCTGCGTTTGGTGG	596
TCP1.4	CTGGACACTCAGCCCTTGG	382
TCP1.5	GGCAGGTGTGCTGGACACT	383
TCP1.6	TTTGATGATGCTGGCGATG	384
TCP1.7	GGGGCTCGTCTTCTACAGG	385
TCP1.8	CAGCAGGAGGATCTTGTAG	386
TCP1.9	TGACCCCAGGAGTGGCACG	387

Primer	Sequence	SEQ ID NO:
TCP1.10	TCAAGCTGACTCGACACCG	388
TCP1.11	CGGCGTGACAGGGCTGC	389
TCP1.12	GCTGAAGGCTGAGTGTCC	390
TCP1.13	TAGTCCATGTTCACAATCG	391

Please replace the paragraph beginning at page 223, line 15, with the following rewritten paragraph:

The cDNA insert of plasmid pGRN121 was sequenced using techniques known in the art. Figure 70 provides a restriction site and function map of plasmid pGRN121 identified based on this preliminary work. The results of this preliminary sequence analysis are shown in Figure 71. From this analysis, and as shown in Figure 70, a putative start site for the coding region was identified at approximately 50 nucleotides from the *Eco*RI site (located at position 707), and the location of the telomerase-specific motifs, "FFYVTE" (SEQ ID NO:361), "PKP," "AYD," "QG", and "DD," were identified, in addition to a putative stop site at nucleotide #3571 (*See,* Figure 72, which shows the DNA and corresponding amino acid sequences for the open reading frames in the sequence ("a", "b", and "c")). However, due to the preliminary nature of the early sequencing work, the reading frames for the various motifs were found not to be in alignment.

Please replace the paragraph beginning at page 224, line 1, with the following rewritten paragraph:

Further sequence analysis resolved the cDNA sequence of pGRN121 to provide a contiguous open reading frame that encodes a protein of molecular weight of approximately 127,000 daltons, and 1132 amino acids as shown in Figure 74. A refined map of pGRN121 based on this analysis, is provided in Figure 73. The results of additional sequence analysis of the hTRT cDNA are presented in Figure 16 SEQ ID NO:1.

Please replace the paragraph beginning at page 224, line 23, with the following rewritten paragraph:

hTRT nucleic acid was amplified from cDNA using oligonucleotide primers Lt5 and Lt6 (Table 2) for a total of 31 cycles (94°C 45s, 60°C 45s, 72°C 90s). GAPDH was amplified using primers K136 (5'-CTCAGACACCATGGGGAA GGTGA; SEQ ID NO:552) and K137 (5'-ATGATCTTGAGGCTGTTGTCATA; SEQ ID NO:553) for a total of 16 cycles (94°C 45 s, 55°C 45 s, 72°C 90 s). hTR was amplified using primers F3b (5'-TCTAACCCTAACTGAGAAGGGCGTAG; SEQ ID NO:597) and R3c (5'-GTTTGCTCTAGAATGAACGGTGGAAG; SEQ ID NO:598) for a total of 22 cycles (94 °C 45s, 55 °C 45 s, 72 °C 90s). TP1 mRNA was amplified using primers TP1.1 and TP1.2 for 28 cycles (cycles the same as hTRT). Reaction products were resolved on an 8% polyacrylamide gel, stained with SYBR Green (Molecular Probes) and visualized by scanning on a Storm 860 (Molecular Dynamics). The results, shown in Figure 5, demonstrate that hTRT mRNA levels correlate directly with telomerase activity levels in the cells tested.

Please replace the paragraph beginning at page 225, line 18, with the following rewritten paragraph:

As shown below, the 104-base intronic sequence SEQ ID NO:7 is inserted in the hTRT mRNA (shown in bold) at the junction corresponding to bases 274 and 275 of Figure 16:
CCCCCCGCCGCCCCCTCCTTCCGCCAG/GTGGGCCTCCCCGGGGTCGGCGTCCGG CTGGGGTTGAGGGCGGCCGGGGGGAACCAGCGACATGCGGAGAGCAGCGCAGGCG ACTCAGGGCGCTTCCCCCGCAG/**GTGTCCTGCCTGAAGGAGCTGGTGGCCCGAGT GCTGCAG** (SEQ ID NO:599)
The "/" indicates the splice junctions; the sequence shows good matches to consensus 5' and 3' splice site sequences typical for human introns.

Please replace the paragraph beginning at page 229, line 7, with the following rewritten paragraph:

This expression vector of the invention is designed for inducible expression in bacteria. The vector can be induced to express, in *E. coli*, high levels of a fusion protein composed of a cleavable, HIS tagged thioredoxin moiety and the full length hTRT protein. The use of the expression system was in substantial accordance with the manufacturer's instructions. The amino acid sequence of the fusion protein encoded by the resulting vector of the invention is shown below; (-*-) denotes an enterokinase cleavage site:

MSDKIIHLTDDSFDTDVLKADGAILVDFWAHWCGPCKMIAPILDEIADEYQGKLTVAKLRIDHN
PGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLAGSGSGDDDDK-*-VPMHEL
EIFEFAAASTQRCVLLRTWEALAPATPAMPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGW
RLVQRGDPAAFRALVAQCLVCVPWDARPPPAAPSFRQVSCLKELVARVLQRLCERGAKNVLAFG
FALLDGARGGPPEAFTTSVRSYLPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVLVAP
SCAYQVCGPPLYQLGAATQARPPPHASGPRRRLGCERAWNHSVREAGVPLGLPAPGARRRGGSA
SRSLPLPKRPRRGAAPEPERTPVGQGSWAHPGRTRGPSDRGFCVVSPARPAEEATSLEGALSGT
RHSHPSVGRQHHAGPPSTSRPPRPWDTPCPPVYAETKHFLYSSGDKEQLRPSFLLSSLRPSLTG
ARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVT
PAAGVCAREKPQGSVAAPEEEDTDPRRLVQLLRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNE
RRFLRNTKKFISLGKHAKLSLQELTWKMSVRDCAWLRRSPGVGCVPAAEHRLREEILAKFLHWL
MSVYVVELLRSFFYVTETTFQKNRLFFYRKSVWSKLQSIGIRQHLKRVQLRELSEAEVRQHREA
RPALLTSRLRFIPKPDGLRPIVNMDYVVGARTFRREKRAERLTSRVKALFSVLNYERARRPGLL
GASVLGLDDIHRAWRTFVLRVRAQDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYC
VRRYAVVQKAAHGHVRKAFKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSG
LFDVFLRFMCHHAVRIRGKSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLRLVD
DFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFPVEDEALGGTAFVQMPAHGLFPWCG
LLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRRKLFGVLRLKCHSLFLDLQVNSLQT
VCTNIYKILLLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTASLCYSILKAKNAGMSLGAKG
AAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQLSRKLPGTTLTALEAAANPALP
SDFKTILD (SEQ ID NO:600)

Please replace the paragraph beginning at page 230, line 10, with the following rewritten paragraph:

To produce large quantities of an hTRT protein fragment, the *E. coli* expression vector pGEX-2TK (Pharmacia Biotech, Piscataway N.J) was selected, and used essentially according to manufacturer's instructions to make an expression vector of the invention. The resulting construct contains an insert derived from nucleotides 3272 to 4177 of the hTRT insert in the plasmid pGRN121. The vector directs expression in *E. coli* of high levels of a fusion protein composed of glutathione-S-transferase sequence (underlined below), thrombin cleavage sequence (double underlined), recognition sequence for heart muscle protein kinase (italicized), residues introduced by cloning in brackets ([GSVTK]; SEQ ID NO:601) and hTRT protein fragment (in bold) as shown below:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVK
LTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPE
MLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGS*RRASV*[GSVTK] **IPQGSILSTLLCSLCYGD**
MENKLFAGIRRDGLLLRLVDDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFPVEDE
ALGGTAFVQMPAHGLFPWCGLLLDTRTLEVQSDYSSYARTSIRASVTFNRGFKAGRNMRRKLFG
VLRLKCHSLFLDLQVNSLQTVCTNIYKILLLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTA
SLCYSILKAKNAGMSLGAKGAAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQLS
RKLPGTTLTALEAAANPALPSDFKTILD (SEQ ID NO:602)

Please replace the paragraph beginning at page 231, line 19, with the following rewritten paragraph:

The vector directs expression in *E coli* of high levels of a fusion protein composed of glutathione-S-transferase sequence (underlined); thrombin cleavage sequence (double underlined); recognition sequence for heart muscle protein kinase (italicized); a set of three and a set of five residues introduced by cloning are in brackets ([GSV] and [GSVTK] SEQ ID NO:601); eight consecutive histidines (also double underlined); and hTRT protein fragment (in bold):

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYI
DGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLK

VDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGS*RRASV*[GSV]H
HHHHHHH[GSVTK]**MSVYVVELLRSFFYVTETTFQKNRLFFYRPSVWSKLQSIGIRQ
HLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKPDGLRPIVNMDYVVGARTFRR
EKRAERLTSRVKALFSVLNYERARRPGLLGASVLGLDDIHRAWRTFVLRVRAQDP
PPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYAVVQKAAHGHVRKA
FKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGLFDVFLRFMCH
HAVRIRGKSYVQCQGI** (SEQ ID NO:603)

Please replace the paragraph beginning at page 232, line 9, with the following rewritten paragraph:

This construct contains an insert derived from nucleotides 2426 to 3274 of the hTRT insert in the plasmid pGRN121, but without the HIS-8 tag of the construct described above. The vector directs expression in *E coli* of high levels of a fusion protein composed of glutathione-S-transferase (underlined), thrombin cleavage sequence (double underlined), recognition sequence for heart muscle protein kinase (italicized), residues introduced by cloning in brackets ([GSVTK]; SEQ ID NO:601) and hTRT protein fragment (in bold):
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVK
LTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPE
MLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGS*RRASV*[GSVTK]**MSVYVVELLRSFFYVTET**
TFQKNRLFFYRPSVWSKLQSIGIRQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKPDGL
RPIVNMDYVVGARTFRREKRAERLTSRKALFSVLNYERARRPGLLGASVLGLDDIHRAWRTFVL
RVRAQDPPPEYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYAVVQKAAHGVRKAFKS
HVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASGLFDVFLRFMCHHAVRIRGKSYV
QCQGI (SEQ ID NO:604)

Please replace the paragraph beginning at page 232, line 28, with the following rewritten paragraph:

This construct contains an insert derived from nucleotides 1625 to 2458 of the hTRT insert in the plasmid pGRN121. The vector directs expression in *E coli* of high levels of a fusion

protein composed of glutathione-S-transferase, (underlined), thrombin cleavage sequence (double underlined), recognition sequence for heart muscle protein kinase (italicized) residues introduced by cloning in brackets ([GSVTK]; SEQ ID NO:601) and hTRT protein fragment (in bold):

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLP
YYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDF
ETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMC
LDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGS***R***
RASV[GSVTK]ATSLEGALSGTRHSHPSVGRQHHAGPPSTSRPPRPWDTPCPPVYAET
KHFLYSSGDKEQLRPSFLLSSLRPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQRY
WQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVTPAAGVCAREKPQGSVAAPEEED
TDPRRLVQLLRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNERRFLRNTKKFISLGK
HAKLSLQELTWKMSVRDCAWLRRSPGVGCVPAAEHRLREEILAKFLHWLMSVYVVE
LLRS (SEQ ID NO:605)

Please replace the paragraph beginning at page 233, line 13, with the following rewritten paragraph:

This construct contains an insert derived from nucleotides 782 to 1636 of the hTRT insert in the plasmid pGRN121. The vector directs expression in *E coli* of high levels of a fusion protein composed of glutathione-S-transferase, (underlined), thrombin cleavage sequence (double underlined), recognition sequence for heart muscle protein kinase (italicized) residues introduced by cloning in brackets ([GSVTK]; SEQ ID NO:601) and hTRT protein fragment (in bold):

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVK
LTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPE
MLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGS*RRASV*[GSVTK]**MPRAPRCRAVRSLLSHYR**
EVLPLATFVRRLGPQGWRLVQRGDPAAFRALVAQCLVCVPWDARPPAAPSFRQVSCLKELVARV
LQRLCERGAKNVLAFGFALLDGARGGPPEATTSVRSYLPNTVTDALRGSGAWGLLLRRVGDDVL
VHLLARCALFVLVAPCAYQVCGPPLYQLGAATQARPPPHASGPRRRLGCERAWNHSVREAGVPL
GLPAPGARRRGGSASRSLPLPKRPRRGAAPEPERTPVGQGSWAHPGRTRGPSDRGFCVVSPARP
AEEATSL (SEQ ID NO:606)

Please replace the paragraph beginning at page 234, line 5, with the following rewritten paragraph:

Specifically, the oligonucleotide 5'- CCGGCCACCCCCCATATGCCGCGCGCTCCC-3' (SEQ ID NO:607) was used as described above to modify hTRT cDNA nucleotides 779 to 781 of the hTRT cDNA (Figure 16) from GCG to CAT. These 3 nucleotides are the last nucleotides before the ATG start codon so they do not modify the protein sequence. The change in sequence results in the creation of a unique NdeI restriction site in the hTRT cDNA. Single-stranded hTRT DNA was used as a DNA source for the site directed mutagenesis. The resulting plasmid was sequenced to confirm the success of the mutagenesis.

Please replace the paragraph beginning at page 236, line 2, with the following rewritten paragraph:

In a further embodiment of the invention, *in vitro* mutagenesis of pGRN125 was done to convert the hTRT initiating ATG codon into a Kozak consensus and create EcoRI and BglII restriction digest sites to facilitate cloning into expression vectors. The oligonucleotide 5'-TGCGCACGTGGGAAGCCCTGGCagatctgAattCcaCcATGCCGCGCGCTCCCCGCTG-3' (SEQ ID NO:608) (altered nucleotides in lower case) was used in the mutagenesis procedure. The resulting expression vector was designated pGRN127.

Please replace the paragraph beginning at page 236, line 9, with the following rewritten paragraph:

In another embodiment of the invention, the second Asp of the TRT "DD motif" was converted to an alanine to create a non-functional telomerse enzyme, thus creating a mutant TRT protein for use as a dominant/negative mutant. The hTRT coding sequence was mutagenized *in vitro* using the oligonucleotide 5'-CGGGACGGGCTGCTCCTGCGTTTGGTGGAcGcgTTCTTGTTGGTGACACCTCACCTCACC-3' (SEQ ID NO:609) to convert the asparagine codon for residue 869 (Asp869) to an alanine

(Ala) codon. This also created an MluI restriction enzyme site. The resulting expression plasmid was designated pGRN130, which also contains the Kozak consensus sequence as described for pGRN127.

Please replace the paragraph beginning at page 237, line 17, with the following rewritten paragraph:

A second *Picha pastoris* expression vector of the invention derived from pPICZ B, also contains the full-length sequence encoding hTRT derived from nucleotides 659 to 4801 of the hTRT insert in the plasmid pGRN121. This hTRT-His6/pPICZ B expression vector encodes full length hTRT protein fused at its C-terminus to the Myc epitope and His6 reporter tag sequences. The hTRT stop codon has been removed and replaced by vector sequences encoding the Myc epitope and the His6 reporter tag as well as a stop codon. This vector is designed to direct high-level inducible expression in yeast of the following fusion protein, which consists of hTRT sequence (underlined), vector sequences in brackets ([L] and [NSAVD]; SEQ ID NO:610) the Myc epitope (double underlined), and the His6 tag (italicized):

MPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGWRLVQRGDPAAFRALVAQCLVCV
PWDARPPPAAPSFRQVSCLKELVARVLQRLCERGAKNVLAFGFALLDGARGGPPEAFTT
SVRSYLPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVLVAPSCAYQVCGPPL
YQLGAATQARPPPHASGPRRRLGCERAWNHSVREAGVPLGLPAPGARRRGGSASRSLP
LPKRPRRGAAPEPERTPVGQGSWAHPGRTRGPSDRGFCVVSPARPAEEATSLEGALSGT
RHSHPSVGRQHHAGPPSTSRPPRPWDTPCPPVYAETKHFLYSSGDKEQLRPSFLLSSLRPS
LTGARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYGVLLKTH
CPLRAAVTPAAGVCAREKPQGSVAAPEEEDTDPRRLVQLLRQHSSPWQVYGFVRACLR
RLVPPGLWGSRHNERRFLRNTKKFISLGKHAKLSLQELTWKMSVRDCAWLRRSPGVGC
VPAAEHRLREEILAKFLHWLMSVYVVELLRSFFYVTETTFQKNRLFFYRKSVWSKLQSI
GIRQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKPDGLRPIVNMDYVVGARTFRR
EKRAERLTSRVKALFSVLNYERARRPGLLGASVLGLDDIHRAWRTFVLRVRAQDPPPEL
YFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYAVVQKAAHGHVRKAFKSHVST
LTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGLFDVFLRFMCHHAVRIRGKS
YVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLRLVDDFLLVTPHLTHAKTFL
RTLVRGVPEYGCVVNLRKTVVNFPVEDEALGGTAFVQMPAHGLFPWCGLLLDTRTLEV
QSDYSSYARTSIRASLTFNRGFKAGRNMRRKLFGVLRLKCHSLFLDLQVNSLQTVCTNI
YKILLLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTASLCYSILKAKNAGMSLGAKG

AAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQLSRKLPGTTLTALEAAA
NPALPSDFKTILD[L]EQKLISEEDL[NSAVD]*HHHHHH* (SEQ ID NO:611)

Please replace the paragraph beginning at page 238, line 35, with the following rewritten paragraph:

A full length hTRT with a His6 and Anti-Xpress tags (Invitrogen) was also constructed. This vector also contains an insert consisting of nucleotides 707 to 4776 of the hTRT insert from the plasmid pGRN121. The vector directs expression in insect cells of high levels of full length hTRT protein fused to a cleavable 6-histidine and Anti-Xpress tags, and the amino acid sequence of the fusion protein is shown below; (-*-) denotes enterokinase cleavage site:

MPRGSHHHHHHGMASMTGGQQMGRDLYDDDDL-*-DPSSRSAAGTMEFAAAST
QRCVLLRTWEALAPATPAMPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGWRLVQ
RGDPAAFRALVAQCLVCVPWDARPPPAAPSFRQVSCLKELVARVLQRLCERGAKNVLA
FGFALLDGARGGPPEAFTTSVRSYLPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARC
ALFVLVAPSCAYQVCGPPLYQLGAATQARPPPHASGPRRRLGCERAWNHSVREAGVPL
GLPAPGARRRGGSASRSLPLPKRPRRGAAPEPERTPVGQGSWAHPGRTRGPSDRGFCVV
SPARPAEEATSLEGALSGTRHSHPSVGRQHHAGPPSTSRPPRPWDTPCPPVYAETKHFLY
SSGDKEQLRPSFLLSSLRPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRPLF
LELLGNHAQCPYGVLLKTHCPLRAAVTPAAGVCAREKPQGSVAAPEEEDTDPRRLVQL
LRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNERRFLRNTKKFISLGKHAKLSLQELT
WKMSVRDCAWLRRSPGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLRSFFYVTETT
FQKNRLFFYRKSVWSKLQSIGIRQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKP
DGLRPIVNMDYVVGARTFRREKRAERLTSRVKALFSVLNYERARRPGLLGASVLGLDDI
HRAWRTFVLRVRAQDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYA
VVQKAAHGHVRKAFKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASS
GLFDVFLRFMCHHAVRIRGKSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLL
LRLVDDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFPVEDEALGGTAFVQ
MPAHGLFPWCGLLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRRKLFGVLRL
KCHSLFLDLQVNSLQTVCTNIYKILLLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTA
SLCYSILKAKNAGMSLGAKGAAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRT
AQTQLSRKLPGTTLTALEAAANPALPSDFKTILD (SEQ ID NO:612)

Please replace the paragraph beginning at page 240, line 6, with the following rewritten paragraph:

pMelBacB directs expression of full length hTRT in insect cells to the extracellular medium through the secretory pathway using the melittin signal sequence. High levels of full

length hTRT are thus secreted. The melittin signal sequence is cleaved upon excretion, but is part of the protein pool that remains intracellularly. For that reason, it is indicated in parentheses in the following sequence. The sequence of the fusion protein encoded by the vector is shown below:

(MKFLVNVALVFMVVYISYIYA)-*-DPSSRSAAGTMEFAAASTQRCVLLRTWEAL
APATPAMPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGWRLVQRGDPAAFRALVA
QCLVCVPWDARPPPAAPSFRQVSCLKELVARVLQRLCERGAKNVLAFGFALLDGARGG
PPEAFTTSVRSYLPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVLVAPSCAY
QVCGPPLYQLGAATQARPPPHASGPRRRLGCERAWNHSVREAGVPLGLPAPGARRRGG
SASRSLPLPKRPRRGAAPEPERTPVGQGSWAHPGRTRGPSDRGFCVVSPARPAEEATSLE
GALSGTRHSHPSVGRQHHAGPPSTSRPPRPWDTPCPPVYAETKHFLYSSGDKEQLRPSFL
LSSLRPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPY
GVLLKTHCPLRAAVTPAAGVCAREKPQGSVAAPEEEDTDPRRLVQLLRQHSSPWQVYG
FVRACLRRLVPPGLWGSRHNERRFLRNTKKFISLGKHAKLSLQELTWKMSVRDCAWLR
RSPGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLRSFFYVTETTFQKNRLFFYRKSV
WSKLQSIGIRQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKPDGLRPIVNMDYVV
GARTFRREKRAERLTSRVKALFSVLNYERARRPGLLGASVLGLDDIHRAWRTFVLRVR
AQDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYAVVQKAAHGHVRK
AFKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGLFDVFLRFMCHH
AVRIRGKSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLRLVDDFLLVTPH
LTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFPVEDEALGGTAFVQMPAHGLFPWCGLL
LDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRRKLFGVLRLKCHSLFLDLQVNS
LQTVCTNIYKILLLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTASLCYSILKAKNAG
MSLGAKGAAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQLSRKLPGTT
LTALEAAANPALPSDFKTILD (SEQ ID NO:613)

Please replace the paragraph beginning at page 244, line 11, with the following rewritten paragraph:

Analysis of telomerase activity using a TRAP assay utilizing the TRAPeze™ Kit (Oncor, Inc., Gaithersburg, MD) showed that transfection of sense hTRT - but not antisense hTRT - generated telomerase activity in both the BJ and IMR90 cell strains.

Please replace the paragraph beginning at page 248, line 30, with the following rewritten paragraph:

The oligonucleotide 5'-TGCGCACGTGGGAAGCCCTGGCagatctgAattCcaCcATGC CGCGCGCTCCCCGCTG-3' (SEQ ID NO:608) was used in *in vitro* mutagenesis of pGRN125 to convert the initiating ATG codon of the hTRT coding sequence into a Kozak consensus sequence and create EcoRI and BglII sites for cloning. Also, oligonucleotide COD2866 was used to convert AmpS to AmpR (ampicillin resistant) and oligonucleotide COD1941 was used to convert CatR (chloramphenicol resistant) to CatS (chloramphenicol sensitive).

Please replace the paragraph beginning at page 249, line 7, with the following rewritten paragraph:

The oligonucleotide 5'-TGCGCACGTGGGAAGCCCTGGCagatctgAattCcaCcATG CCGCGCGCTCCCCGCTG-3' (SEQ ID NO:608) is used in *in vitro* mutagenesis to convert the initiating ATG codon of hTRT into a Kozak consensus and create EcoRI and BglII sites for cloning. Also, oligo 5'-CTGCCCTCAGACTTCAAGACCATCCTGGACTACAA GGACGACGATGACAAATGAATTCAGATCTGCGGCCGCCACCGCGGTGGAGCTCCAG C-3' (SEQ ID NO:614) is used to insert the IBI Flag (International Biotechnologies Inc. (IBI), Kodak, New Haven, CT) at the C-terminus and create EcoRI and BglII sites for cloning. Also, COD2866 is used to convert AmpS to AmpR and COD1941 is used to convert CatR to CatS.

Please replace the paragraph beginning at page 249, line 17, with the following rewritten paragraph:

The oligonucleotide 5'-CGGGACGGGCTGCTCCTGCGTTTGGTGGAcGcgTTCTTG TTGGTGACACCTCACCTCACC-3' (SEQ ID NO:609) was used by *in vitro* mutagenesis to convert Asp869 to an Ala codon (i.e. the second Asp of the DD motif was converted to an Alanine to create a dominant/negative hTRT mutant). This also created a MluI site. Also, oligonucleotide 5'-CTGCCCTCAGACTTCAAGACCATCCTGGACTACAAGG ACGACGATGACAAATGAATTCAGATCTGCGGCCGCCACCGCGGTGGAGCTCCAGC-3' SEQ ID NO:614) was used to insert the IBI Flag at the C-terminus and create EcoRI and BglII

sites for cloning. Also, COD2866 was used to convert AmpS to AmpR and COD1941 was used to convert CatR to CatS.

Please replace the paragraph beginning at page 249, line 28, with the following rewritten paragraph:

The oligonucleotide 5'-CGGGACGGGCTGCTCCTGCGTTTGGTGGAcGcgTTCTT GTTGGTGACACCTCACCTCACC-3' (SEQ ID NO:609) was used in *in vitro* mutagenesis to convert the Asp869 codon into an Ala codon (i.e. the second Asp of the DD motif was converted to an Alanine to make a dominant/negative variant protein). This also created an MluI site. Also, the oligonucleotide 5'-TGCGCACGTGGGAAGCCCTGGCagatctgAatt CcaCcATGCCGCGCGCTCCCCGCTG-3' (SEQ ID NO:608) was used in *in vitro* mutagenesis to convert the initiating ATG codon of the hTRT coding sequence into a Kozak consensus sequence and create EcoRI and BglII sites for cloning. Also, COD2866 was used to convert AmpS to AmpR and COD1941 was used to convert CatR.

Please replace the paragraph beginning at page 251, line 22, with the following rewritten paragraph:

The oligonucleotide 5'- CTGCCCTCAGACTTCAAGACCATCCTGGACTACAAGG ACGACGATGACAAATGAATTCAGATCTGCGGCCGCCACCGCGGTGGAGCTCCAGC-3' (SEQ ID NO:614) was used to insert the IBI Flag at the C-terminus of hTRT in pGRN125 and create EcoRI and BglII sites for cloning. Also, COD2866 was used to convert AmpS to AmpR and COD1941 was used to convert CatR to CatS.

Please replace the paragraph beginning at page 253, line 13, with the following rewritten paragraph:

This vector is an intermediate vector for constructing a hTRT fusion protein expression vector. The mutagenic oligo 5'-cttcaagaccatcctggactttcgaaacgcggccgccaccg

cggtggagctcc-3' (SEQ ID NO:615) was used to add a CSP45I site at the C-terminus of hTRT by *in vitro* mutagenesis of pGRN125. The "stop" codon of hTRT was deleted and replaced with a Csp45I site. The selectable marker that is used with this vector is ampicillin.

Please replace the paragraph beginning at page 256, line 20, with the following rewritten paragraph:

This vector was constructed for the expression and mutagenesis of TRT sequences in *E. coli*. The coding sequence is driven by a T7 promoter. Oligonucleotide RA45 (5'-GCCACCCCCGCGCTGCCTCGAGCTCCCCGCTGC-3'; SEQ ID NO:616) is used in *in vitro* mutagenesis to change the initiating met in hTRT to Leu and introduce an XhoI site in the next two codons after the Leu. Also COD 1941 was used to change CatR to CatS, and introduces a BSPH1 site, and COD 2866 was used to change AmpS to AmpR, introducing an FSP1 site. The selectable marker used with this vector is amphicillin.

Please replace the paragraph beginning at page 256, line 29, with the following rewritten paragraph:

This vector was constructed for the expression of hTR sequences in *E. coli*. Primers hTR+1 5'-GGGGAAGCTTTAATACGACTCACTATAGGGTTGCGGAGGGTGG GCCTG-3' (SEQ ID NO:617) and hTR+445 5'-CCCCGGATCCTGCGCATGTGTGAGCCGAGTCCT GGG-3' (SEQ ID NO:618) were used to amplify by PCR a fragment from pGRN33 containing the full length hTR with the T7 promoter on the 5' end (as in hTR+1). A BamHI-HindIII digest of the PCR product was put into the BamHI-HindIII sites of pUC119. The coding sequence operably linked to a T7 promoter. The selectable marker used with this vector is amphicillin. pGRN164 is also called phTR+1.

Please replace the paragraph beginning at page 259, line 27, with the following rewritten paragraph:

As shown in Figure 10, reticulocyte lysate alone has no detectable telomerase activity (lane 6). Similarly, no detectable activity is observed when either hTR alone (lane 1) or full length hTRT gene (lane 4) are added to the lysate. When both components are added (lane 2), telomerase activity is generated as demonstrated by the characteristic repeat ladder pattern. When the carboxyl-terminal region of the hTRT gene is removed by digestion of the vector with *Nco*I ("truncated hTRT") telomerase activity is abolished (lane 3). Lane 5 shows that translation of the truncated hTRT alone does not generate telomerase activity. Lane "R8" shows a positive control for a telomerase product ladder generated by TRAP of TSR8, a synthetic telomerase product having a nucleotide sequence of 5'-ATTCCGTCGAGCAGAGTTAG[GGTTAG]$_7$-3' (SEQ ID NO:619).

Please replace the paragraph beginning at page 261, line 9, with the following rewritten paragraph:

Telomerase activity was assayed in a two step procedure. In step one, a conventional telomerase assay was performed as described in Morin, 1989, *Cell* 59: 521, except no radiolabel was used. In step two, an aliquot was assayed by the TRAP procedure for 20-30 cycles as described *supra*. The conventional assay was performed by assaying 1-10 µl of reconstituted telomerase in 40-50 µl final volume of 25 mM Tris-HCl, pH 8.3, 50 mM K-acetate, 1 mM EGTA, 1 mM $MgCl_2$, 2 mM dATP, 2 mM TTP, 10 uM dGTP, and 1 uM primer (usually M2, 5'-AATCCGTCGAGCAGAGTT; SEQ ID NO:620) at 30° C for 60-180 minutes. The reaction was stopped by heating to 95° C for 5 minutes and 1-10 µl of the first step mixture was carried onto the step two TRAP reaction (50 ul).

Please replace the paragraph beginning at page 262, line 25, with the following rewritten paragraph:

The following example demonstrates that *in vitro* reconstituted (IVR) telomerase can be assayed using conventional telomerase assays in addition to amplification-based assays (i.e., TRAP). IVR telomerase as described in part (B), *supra* (the "linked reconstitution method")

followed by DEAE purification, as described *supra* was assayed using the gel blot assay using the following reaction conditions; 1-10 µl of linked IVR telomerase in 40 µl final volume of 25 mM Tris-HCl, pH 8.3, 50 mM K-acetate, 1 mM EGTA, 1 mM $MgCl_2$, 0.8 mM dATP, 0.8 mM TTP, 1.0 mM dGTP, and 1 uM primer (M2, *supra*; or H3.03, 5'-TTAGGGTTAGGGTTAGGG; SEQ ID NO:621) at 30°C for 180 minutes. The telomeric DNA synthesized was isolated by standard procedures, separated on a 8 % polyacrylamide, 8 M urea gel, transferred to a nylon membrane, and probed using the ^{32}P-(CCCTAA)n riboprobe used in the dot-blot assay. The probe identified a six nucleotide ladder in the lane representing 10 µl of IVR telomerase that was equivalent to the ladder observed for 5 µl of native nuclear telomerase purified by mono Q and heparin chromatography. The results show that IVR telomerase possesses processive telomerase catalytic activity equivalent to native telomerase.

Please replace the paragraph beginning at page 263, line 26, with the following rewritten paragraph:

This experiment demonstrates that IVR telomerase recognizes primer 3' termini equivalently to native (purified) telomerase. Telomerase forms a base-paired duplex between the primer 3' end and the template region of hTR and adds the next specified nucleotide (Morin, 1989, *supra*). To verify that IVR (recombinant) telomerase has the same property, the reactions of primers with ---GGG or ---TAG 3' termini (AATCCGTCGAGCAGAGGG; SEQ ID NO:622 and AATCCGTCGAGCAGATAG; SEQ ID NO:623) were compared to a primer having a ---GTT 3' terminus (M2 *supra*) using IVR and native telomerase assayed by the two step conventional/TRAP assay detailed above. The product ladders of the ---GGG and ---TAG primers were shifted +4 and +2, respectively, when compared to the standard primer (---GTT 3' end), the same effect as was observed with native telomerase. This experiment demonstrates IVR and native telomerases recognize primer termini in a similar manner.

Please replace the paragraph beginning at page 264, line 14, with the following rewritten paragraph:

To produce anti-hTRT antibodies, the following peptides from hTRT were synthesized with the addition of C (cysteine) as the amino terminal residue (see Figure 54).

S-1: FFY VTE TTF QKN RLF FYR KSV WSK	SEQ ID NO:232
S-2: RQH LKR VQL RDV SEA EVR QHR EA	SEQ ID NO:233
S-3: ART FRR EKR AER LTS RVK ALF SVL NYE	SEQ ID NO:234
A-3: PAL LTS RLR FIP KPD GLR PIV NMD YVV	SEQ ID NO:237

Please replace TABLE 8 with the following rewritten table:

TABLE 8

Fragment	Primer Set 1	Primer Set 2	Final Size	Primers for Seq
OA	na	K320/K322	208	K320,K322
A	K320/TCP1.43	TCP1.40/ TCP1.34	556	TCP1.52, TCP1.39, K322, TCP1.40, TCP1.41, TCP1.30, TCP1.34, TCP1.49
B	TCP1.42/ TCP1.32B	TCP1.35/ TCP1.21	492	TCP1.35, TCP1.28, TCP1.38, TCP1.21, TCP1.46, TCP1.33, TCP1.48
C	TCP1.65/ TCP1.66	TCP1.67/ TCP1.68	818	TCP1.67, TCP1.32, TCP1.69, TCP1.68, TCP1.24, TCP1.44, K303
D2	K304/billTCP6	Lt1/TCP1.6	546	Lt2, Lt1, TCP1.6, billTCP4, TCP1.13, TCP1.77, TCP1.1
D3	TCP1.12/ TCP1.7	TCP1.14/ TCP1.15	604	TCP1.6, TCP1.14, TCP1.73, TCP1.78, TCP1.25, TCP1.15, TCP1.76
EF	na	TCP1.74/ TCP1.7	201	TCP1.74, TCP1.7, TCP1.75, TCP1.15, TCP1.3
E	TCP1.3/ TCP1.4	TCP1.2/ TCP1.9	687	TCP1.2, TCP1.8, TCP1.9, TCP1.26
F	TCP1.26/UTR2	TCP1.10 TCP1.4	377	TCP1.4, TCP1.10, TCP1.11

Please replace the paragraph beginning at page 270, line 2, with the following rewritten paragraph:

For generation of the first RNAse protection probe, the PCR product from the following primer pair (T701 and reverse01) is used:
T701 5'-GGGAGATCT TAATACGACTCACTATAG ATTCA GGCCATGGTG CTGCGCCGGC TGTCA GGCTCCC ACGACGTAGT CCATGTTCAC-3' (SEQ ID NO:624); and reverse01 5'-GGGTCTAGAT CCGGAAGAGTGT CTGGAGCAAG-3' (SEQ ID NO:625).

Please replace the paragraph beginning at page 274, line 30, with the following rewritten paragraph:

To identify sequences or elements that play a role in hTRT expression, expression was tested using promoter-reporter constructs with varying amounts of the upstream region (5' to the transcription initiation site) of the hTRT gene. Experiments were conducted using pGRN 150 [which contains approximately 2405 bp of genomic sequence upstream of the most 5' nucleotide present in the hTRT cDNA], pGRN 176 [which contains approximately 186 bp of genomic sequence upstream of the most 5' nucleotide present in the hTRT cDNA] and pGRN 175 [which contains approximately 77 bp of genomic sequence upstream of the most 5' nucleotide present in the hTRT cDNA]. The following sequence is present in pGRN 176 but not pGRN 175: 5'-GTGGCGGAGGGACTGGGGACCCGGGC ACCGGTCCTGCCCCTTCACCTTCCAGCTCCGCCTCGTCCGCGCGGAACCCCGCCCCG TCCCGAACCCTTCCCGGGTCCCCGGCCCAGCCCCTTCCGGG-3' (SEQ ID NO:726).

Please replace the paragraph beginning at page 276, line 21, with the following rewritten paragraph:

A vector for expression of an hTRT-EGFP fusion protein in mammalian cells was constructed by placing the EcoRI insert from pGRN124 (see Example 6) into the EcoRI site of pEGFP-C2 (Clontech, San Diego, CA). The amino acid sequence of the fusion protein is

provided below. EGFP residues are in bold, residues encoded by the 5' untranslated region of hTRT mRNA are underlined, and the hTRT protein sequence is in normal font.

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPT
LVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTL
VNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLA
DHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKS
GRTQISSSSFEFAAASTQRCVLLRTWEALAPATPAMPRAPRCRAVRSLLRSHYREVLPLA
TFVRRLGPQGWRLVQRGDPAAFRALVAQCLVCVPWDARPPPAAPSFRQVSCLKELVARVL
QRLCERGAKNVLAFGFALLDGARGGPPEAFTTSVRSYLPNTVTDALRGSGAWGLLLRRVG
DDVLVHLLARCALFVLVAPSCAYQVCGPPLYQLGAATQARPPPHASGPRRRLGCERAWNH
SVREAGVPLGLPAPGARRRGGSASRSLPLPKRPRRGAAPEPERTPVGQGSWAHPGRTRGP
SDRGFCVVSPARPAEEATSLEGALSGTRHSHPSVGRQHHAGPPSTSRPPRPWDTPCPPVY
AETKHFLYSSGDKEQLRPSFLLSSLRPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQR
YWQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVTPAAGVCAREKPQGSVAAPEEEDTDP
RRLVQLLRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNERRFLRNTKKFISLGKHAKLSL
QELTWKMSVRDCAWLRRSPGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLRSFFYVTE
TTFQKNRLFFYRPSVWSKLQSIGIRQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIP
KPDGLRPIVNMDYVVGARTFRREKRAERLTSRVKALFSVLNYERARRPGLLGASVLGLDD
IHRAWRTFVLRVRAQDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYA
VVQKAAHGHVRKAFKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGL
FDVFLRFMCHHAVRIRGKSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLR
LVDDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFPVEDEALGGTAFVQMPAH
GLFPWCGLLLDTRTLEVQSDYSSYARTSIRASVTFNRGFKAGRNMRRKLFGVLRLKCHSL
FLDLQVNSLQTVCTNIYKILLLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTASLCYS
ILKAKNAGMSLGAKGAAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQLSR
KLPGTTLTALEAAANPALPSDFKTILD (SEQ ID NO:628)

Please replace the paragraph beginning at page 278, line 3, with the following rewritten paragraph:

In other applications, regions of the hTRT can be mutagenized to identify regions (e.g., residues 193-196 (PRRR; SEQ ID NO:541) and 235-240 (PKRPRR; SEQ ID NO:542)) required for nuclear localization, which are targets for anti-telomerase drugs (telomerase activity modulators). Other applications include:

use of the fusion protein as a fluorescent marker of efficient cell transfection for both transient transfection experiments and when establishing stable cell lines expressing EGFP-hTRT;

expression of an hTRT-EGFP fusion with mutated nuclear localization signals (deficient for nuclear localization) in immortal cells so that the hTRT mutant-EGFP scavenges all the hTR of the immortal cells, retaining it in the cytoplasm and preventing telomere maintenance; and

use as a tagged protein for immunoprecipitation.

Please replace the paragraph beginning at page 278, line 29, with the following rewritten paragraph:

A. **Mutation of hTRT FFYxTE (SEQ ID NO:360) Motif**

Please replace the paragraph beginning at page 278, line 30, with the following rewritten paragraph:

In initial experiments, a vector encoding an hTRT mutant protein, "F560A," was produced in which amino acid 560 of hTRT was changed from phenylalanine (F) to alanine (A) by site directed mutagenesis of pGRN121 using standard techniques. This mutation disrupts the TRT FFYxTE (SEQ ID NO:360) motif. The resulting F560A mutant polynucleotide was shown to direct synthesis of a full length hTRT protein as assessed using a cell-free reticulocyte lysate transcription/translation system in the presence of ^{35}S-methionine.

Please replace the paragraph beginning at page 279, line 27, with the following rewritten paragraph:

Amino acid alignment of the known TRTs identified a telomerase-specific motif, motif T (see *supra*). To determine the catalytic role of this motif in hTRT, a six amino acid deletion in this motif (Δ560-565; FFYxTE; SEQ ID NO:360), was constructed using standard site directed mutagenesis techniques (Ausubel, *supra*). The deletion was assayed using IVR telomerase using the two step conventional/TRAP assay detailed in Example 7. The Δ560-565 mutant had no observable telomerase activity after 25 cycles of PCR whereas wild type hTRT IVR telomerase produced a strong signal. Each amino acid in each residue in motif T was examined independently in a similar manner; mutants F560A, Y562A, T564A, and E565A retained intermediate levels of telomerase activity, while a control mutant, F487A, had minimal affect on

activity. Notably, mutant F561A had greatly reduced or undetectable telomerase activity, while activity was fully restored in its "revertant", F561A561F. F561A561Fchanges the mutated position back to its original phenylalanine. This is a control that demonstrates that no other amino acid changes occurred to the plasmid that could account for the decreased activity observed. Thus, the T motif is the first non-RT motif shown to be absolutely required for telomerase activity.

Please replace the paragraph beginning at page 281, line 17, with the following rewritten paragraph:

The recombinant telomerase product (IVRP) is assayed in the presence and absence of multiple concentrations of test compounds which are solubilized in DMSO (e.g. 10 μM - 100 μM). Test compounds are preincubated in a total volume of 25 μL for 30 minutes at room temperature in the presence of 2.5 μL IVRP, 2.5% DMSO, and 1X TRAP Buffer (20 mM Tris-HCl, pH 8.3, 1.5mM $MgCl_2$, 63 mM KCl, 0.05%Tween20, 1.0 mM EGTA, 0.1 mg/ml Bovine serum albumin). Following the preincubation, 25 μL of the TRAP assay reaction mixture is added to each sample. The TRAP assay reaction mixture is composed of 1X TRAP buffer, 50μL dNTP, 2.0 μg/ml primer ACX, 4 μg/ml primer U2, 0.8 attomol/ml TSU2, 2 units/50μl Taq polymerase (Perkin Elmer), and 2 μg/ml [^{32}P]5'end-labeled primer TS (3000Ci/mmol). The reaction tubes are then placed in the PCR thermocycler (MJ Research) and PCR is performed as follows: 60 min at 30°C, 20 cycles of {30 sec at 94°C, 30 sec. at 60°C, 30 sec. at 72°C}, 1 min at 72°C, cool down to 10°C. The TRAP assay is described, as noted *supra*, in U.S. Patent No. 5,629,154. The primers and substrate used have the sequences: TS Primer (5'-AATCCGTCGAGCAGAGTT-3'; SEQ ID NO:629); ACX Primer (5'-GCGCGG[CTTACC]$_3$CTAACC-3'; SEQ ID NO:630); U2 primer (5'-ATCGCTTCTCGGCCTTTT-3'; SEQ ID NO:631); TSU2 (5'-AATCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT-3'; SEQ ID NO:632)